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Autosomal Dominant Polycystic Kidney Disease (ADPKD) is caused by the dysregulation of the PKD1 or PKD2 genes. Among the multiple molecular and biological changes associated with the cystogenic conversion are the amplification of the centrosome, genomic instability and aneuploidy, as well as an increase in the expression of the adhesion molecule integrinβ1. The scope of the study is to elucidate in the molecular mechanism underlying these events and the role of Intβ1 in ADPKD pathogenesis. Our recent work has confirmed that Intβ1 inhibition reverses the centrosome amplification and the increased fibronectin deposition that distinguish cystic as well PC1 knockdown cells. We have also characterized a novel cell line from human loop of Henle epithelium that can serve as a unique model to study medullary cystic kidney disease-2 (MCKD2) and familial juvenile hyperuricemic nephropathy (FJHN). MCKD2 and FJHN are autosomal dominant diseases with renal cystic involvement that are caused by aberrant function of the ciliary uromodulin gene. Using this model we have observed similar centrosomal amplification and genetic instability upon knockdown of uromodulin, suggesting that these biological changes may be a common denominator of the renal cystic phenotype. Breeding of the mice with floxed Pkd1 and Itgb1 (Intβ1) genes to be crossed with the specific transgenic mouse expressing the Cre recombinase under the control of the kidney-restricted Aqp2 promoter is progressing on schedule.

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Kidney, integrin, cyst, polycystic, siRNA, cell cycle, centrosome, uromodulin

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## Table of Contents

**Page**

## INTRODUCTION

Our previous findings indicated that fluctuations of expression of PC1, the product of the *Pkd1* gene, from its physiological range causes centrosome amplification, genetic instability and the emergence of genotypic heterogeneity. Based on our observations, we proposed a genomic instability model of cystogenesis. This model is supported by the evidence that cysts form in a relatively low number of nephrons and that apoptotic cells are present in both tubular and cystic cells in ADPKD kidneys, and is consistent with the clonality of cystic epithelia. Our model predicts the great variability of ADPKD manifestations observed among individuals sharing identical germline mutations and provides a PC1-dependent mechanism for the generation of somatic mutation that may trigger cyst initiation [1]. Since our first description of the role of PC1 expression on centrosome integrity, similar effects have been observed by us upon downregulation of *Pkd2* gene, and by others following the inhibition of other cystic genes, the autosomal recessive polycystic kidney disease gene, *Phkd1* (fibrocystin/polyductin), and the Meckel-Gruber syndrome genes, *Mks1* and *Mks3* [2,3]. Genomically aberrant cells are usually eliminated through mitotic catastrophe or apoptosis. Nevertheless, similarly to carcinogenic transformation, some cells may escape negative selection at low frequency and in a stochastic fashion, thus allowing the emergence of the cystic phenotype.

The molecular mechanisms driving the cystic transformation remain unknown and likely involve molecules that support the survival of genetically abnormal cells. In agreement with in vivo observations, we showed that integrin-$\alpha$2 and integrin-$\beta$1 (Int$\beta$1) are upregulated after the knockdown of *Pkd1* in mouse cells and that integrin-$\alpha2\beta1$ mediated the resistance of *Pkd1* knockdown (*Pkd1*$^{kd}$) cells to *anoikis* [4].

**We hypothesized that Intβ1 is a necessary mediator of cystogenesis. The objective of the application is to dissect the role of Intβ1 in the control of centrosome integrity, the genomic instability and the cystogenetic progression pathways triggered by the loss of PC1.** We proposed that Int$\beta$1 plays a protective role in the genetic adaptation of the PC1 deficient cells allowing the survival of the cystogenic phenotype.

The identification of the factors promoting the adaptation of *Pkd1* defective cells and the elucidation of their effector mechanisms will provide a better understanding of the pathogenesis of renal cystic diseases and help identify novel potential therapeutic targets.

## BODY

*Establishment of a human renal epithelial cell line.* In order to generate a human model to study the role of Int$\beta$1 in cystic development, we engineered a lentiviral vector for the expression of the human telomerase reverse transcriptase (hTERT) (VVPW/Bleo/hTERT) and used it to immortalize primary human renal epithelial cells with the purpose to isolate a stable cell line with characteristics of the distal tubules and collecting duct cells.

We initially screened the clones according to their karyogram with the rationale that clones with a near-normal genetic organization would more faithfully behave as primary cells. Of the initial eight clones that we obtained, one clone, hereafter referred to as TIRE131, presented a seemingly normal 46-chromosome male karyotype and was therefore subjected to further characterization. Southern hybridization of genomic TIRE131 DNA indicated that it contained 2 copies of the VVPW/Bleo/hTERT lentivirus used for immortalizing the primary epithelial cells. Comparative genomic hybridization performed on TIRE131 cells identified a near normal karyotype with an amplified region on the long arm of chromosome 5 and a deletion of one long arm of chromosome 11. These findings were further confirmed by spectral karyotyping.

Morphologically, TIRE131 monolayers displayed a characteristic epithelial appearance. To better define their anatomical origin, we used markers specific for different segments of the nephron. TIRE131 stained positively for the epithelial antigens cytokeratin-8 and for cytokeratin-19. The latter is a member of the cytokeratin family that is prevalently present on cells of the renal distal tubules and collecting ducts, suggesting that TIRE131 originated from distal nephron. Indeed, no expression of markers of proximal tubule, such as alkaline phosphatase and AQP1 antigen, was detected in TIRE131 cells. TIRE131 cells

appeared to stain very weakly for AQP2 but were highly positive for uromodulin (Tamm-Horsfall antigen). These findings were confirmed by immunoblot and strongly suggested that TIRE131 cells originated from the thick ascending limb of the loop of Henle.

Though we were originally trying to derive a collecting duct cell line, these results presented the unique opportunity to study the biology of uromodulin, the product of the UMOD gene whose mutations account for other autosomal dominant disorders with renal cystic involvement: medullary cystic kidney disease-2 (MCKD2) and familial juvenile hyperuricemic nephropathy (FJHN).

Therefore, we further characterized the TIRE131 clone to confirm their loop of Henle origin. Similarly to the loop of Henle epithelium, the TIRE131 cells: 1) possessed a significant resistance to hyperosmotic growth conditions; 2) formed a functional epithelium with tight junction and apicobasal polarization as indicated by the apical staining with ZO1, a marker of tight junction; and 3) displayed a progressive and significant increase in the transepithelial electric resistance (TER) when cultured on transwell. Moreover, TIRE131 cells maintained the tubulocystogenic potential, when grown in collagen type I matrix with clearly detectable lumina in some of the tubular segments and in cystic formations, some of which displayed cilia on their apical side facing the lumen. Uromodulin was found to localize on the cilia of TIRE1 cells, in agreement with previous observations.

To our knowledge, this is the first human cell line derived from the thick ascending limb of the loop of Henle and our data indicated that TIRE131 may be a useful model to study the pathogenetic mechanisms of uromodulin-associated kidney diseases. Further studies indicated that inhibition of uromodulin expression in TIRE131 lead to centrosome amplification, genomic instability, and cystic growth in three-dimensional collagene culture, thus supporting the notion that these events may be common to all renal cystic diseases.

These data have been presented at the last American Society of Nephrology 43$^{rd}$ Annual Meeting, 2010, (Battini *et al*, American Society of Nephrology 43$^{rd}$ Annual Meeting, 2010, Poster # SA-PO2455, Appendix 1) and a manuscript detailing these results is under preparation.
We are planning to explore the effects of uromodulin suppression on the Int$\beta$1 and matrix protein expression to determine whether this mechanism is conserved in the cystic phenotype independently of the causative gene.

In the meantime, from a second round of cloning of the hTERT immortalized human renal epithelial cells, we have also succeeded in isolating a clone that is negative for alkaline phosphatase activity but highly resistant to growth in hyperosmolar conditions and displaying high AQP2 expression levels. We think that this clone likely originates from collecting ducts and we are currently characterizing its functional properties further.

*Inhibition of Int$\beta$1 reverts centrosome amplification and aberrant matrix deposition.* We have shown that the suppression of Int$\beta$1 expression during the inactivation of Pkd1 not only inhibits the hyperproliferative nature of the PC1 knockdown cells, but also significantly reduces the number of cells with increased centrosome amplification and the deposition of fibronectin (Lee *et al*, American Society of Nephrology 43$^{rd}$ Annual Meeting, 2010, Poster # F-PO1769, Appendix 2).

Experiments with time-lapse microscopy that are necessary to assess whether the centrosome defects are a result of the decreased rate of cell division or the direct effect of the reduced Int$\beta$1 expression have been delayed by a mycoplasma contamination of our murine cell lines. We have performed extensive tests and we have now re-derived all the cell lines from clean batches and we are currently assessing them for characteristic biological responses (e.g. growth and Int$\beta$1 overexpression upon Pkd1 knockdown) before proceeding with the live imaging.

*In vivo analysis of Intβ1 cystogenic role: mouse breeding strategy.* To overcome the early lethality associated with the loss of Pkd1 or Itgb1 (Intβ1) genes, we originally proposed to generate an inducible Intβ1 knockdown mouse model to determine the cystogenic role of integrin-β1 *in vivo*. This strategy relies on the time-consuming characterization of an entirely new mouse model. Since the time of the proposal, mouse strains with floxed Pkd1 or Itgb1, as well as a transgenic mouse in which the expression of the Cre recombinase is under the control of the kidney-specific Aqp2 promoter have become commercially available, thus offering an the possibility to more quickly address the function of Intβ1 in cystic kidneys. Therefore, we have taken the approach of conditionally inactivating *Pkd1* and *Itgb1* genes specifically in the kidney by generating mice homozygous for the floxed *Pkd1*, *Itgb1* alleles and carrying the kidney-specific AQP2-cre transgene (*Pkd1*$^{fl/fl}$, *Itgb1*$^{fl/fl}$; AQP2-cre).

To generate *Pkd1*$^{fl/fl}$, *Itgb1*$^{fl/fl}$; AQP2-cre mice, from Jackson Laboratory three murine strains were obtained carrying either *Pkd1*$^{fl/fl}$ (B6.129S4-*Pkd1*$^{tm2Ggg}$/J), the *Itgb1*$^{fl/fl}$ allele (B6;129-*Itgb1*$^{tm1Efu}$/J), or the hemizygous AQP2-cre transgene (B6.Cg-Tg(Aqp2-cre)1Dek/J). The *Pkd1*$^{fl/fl}$ mice possess *loxP* sites in introns 1 and 4 of the *Pkd1* gene [5]; the *Itgb1*$^{fl/fl}$ mice possess *loxP* sites on either side of exon 3 of the *Itgb1* gene. Both strains of mice have been used to generate effective null alleles when bred with cre transgenic mouse [6,7]. The *cre* activity in Aqp2-cre transgenic mouse, directed by the mouse aquaporin 2 promoter, has been observed in kidney collecting duct cells and in testes [7]. Therefore, our breeding scheme is devised to ensure maternal inheritance of the transgene, since paternal inheritance would result in gene recombination not only in the kidney, but also in sperm and the resulting fertilized oocytes. Our breeding scheme is briefly outlined in Figure 1. We currently have obtained the F2 generation of newborn mice and expect to have the F3 generation of mice ready for analysis in the next few months.

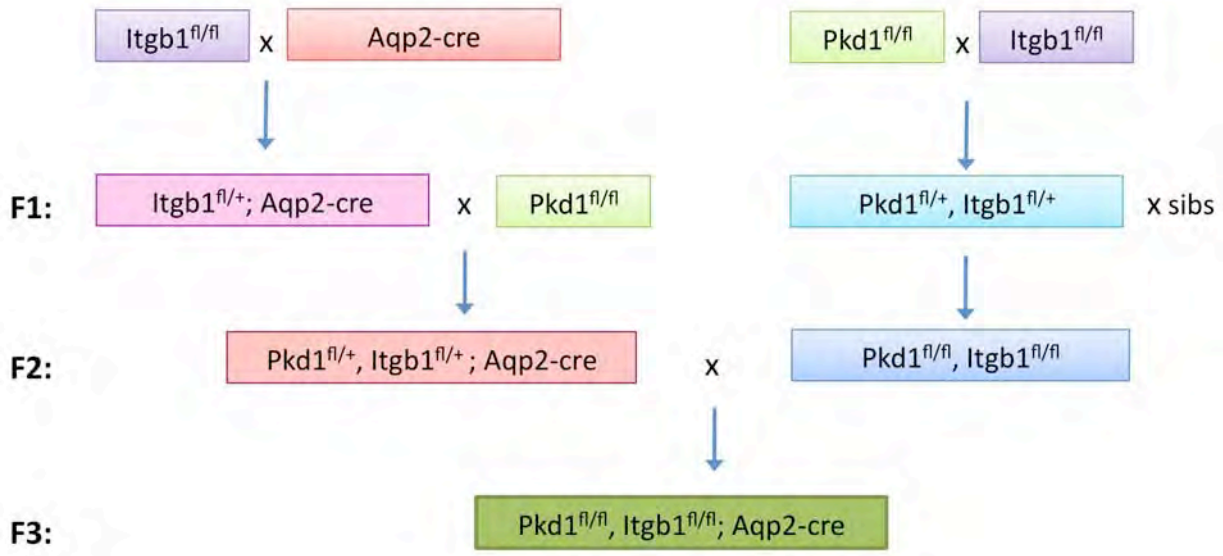



***Figure1.*** *Scheme of the crossings required for the generation of the Pkd1-Intβ1 double kidney knock out. Maternal inheritance is required in association with the Cre transgene in F2.*

*Establishment of murine Pkd1*$^{fl/fl}$ *cell lines.* We have constructed two lentiviral vectors expressing the murine mTert gene or the temperature sensitive SV40 large T antigen. These vectors have been used for the transduction of epithelial cells isolated from the renal corticomeduallary region and papillae of *Pkd1*$^{fl/+}$ and *Pkd1*$^{fl/null}$ mice derived from the crossing of the *Pkd1*$^{fl/fl}$ mice with the heterozygous *Pkd1*$^{null/+}$ strain [8]. These cells are currently being expanded and will be then functionally characterized to produce clonal populations of distal tubule and collecting duct cells. To achieve the complete conditional inactivation/knock out of the *Pkd1* gene in vitro, the floxed region of the gene will be excised upon transduction with a lentivector co-expressing the Cre recombinase and a fluorescent histone 2B-Cherry red (H2B-Cherry) chimeric traceable gene that we have recently engineered. To prevent possible adverse effects of the constitutive expression of the Cre recombinase, this lentivector will be packaged

using an integrase-defective complementary plasmids that does not allow the integration of the provirus while permitting the transient expression of the Cre and the marker H2B-Cherry protein. Lack of stable Cre expression will be tested by observing the disappearance of the H2B-Cherry protein and confirmed by PCR. This system will allow us to study the early events of *Pkd1* loss in cells with normal or knockdown expression of Intβ1.

## KEY RESEARCH ACCOMPLISHMENTS

Established the first human cell line from the loop of Henle epithelial cells.
Demonstrated the role of uromodulin in the control of centrosome integrity and genomic stability.
Proved that the suppression of Intβ1 reverses centrosome amplification and fibronectin expression in Pkd1 knockdown cells.

## REPORTABLE OUTCOMES

The current studies produced the following presented/published papers:

- Uromodulin Controls Centrosome Integrity in Cells of the Thick Ascending Limb of the Loop of Henle. Lorenzo Battini, Kim Lee, Carles Martinez-Romero, Lin Geng, and G. Luca Gusella. American Society of Nephrology 43rd Annual Meeting, 2010, Poster #SA-PO2455.
- Role of Integrin-β1 in cystogenesis and hyperproliferation of ADPKD cells. Kim Lee, Lorenzo Battini, Carles Martinez-Romero, Lin Geng, and G. Luca Gusella. American Society of Nephrology 43rd Annual Meeting, 2010, Poster #F-PO1769.
- Blitzer A.L., Panagis L., Gusella G.L., Danias J., Mlodzik M., and Iomini, C.: Primary cilia dynamics instruct tissue patterning and repair of corneal endothelium. *Proc. Natl. Acad. Sci. U.S.A.* 108(7):2819-24, 2011.
- Lee K., Battini L., and Gusella G.L. Cilium, centrosome and cell cycle regulation in polycystic kidneys disease. *Biochim. Biophys. Acta*, 2011, *In press*.

## CONCLUSIONS

Centrosome amplification and genetic instability seem to be common events in cystic models independently of the cystic gene involved.
Increased Intβ1 expression in *Pkd1* knockdown cells is required for centrosomal amplification and changes in matrix deposition.
The role of Intβ1 in these events suggests that targeting Intβ1 could be potentially beneficial to interfere with the progression of the renal cystogenesis.

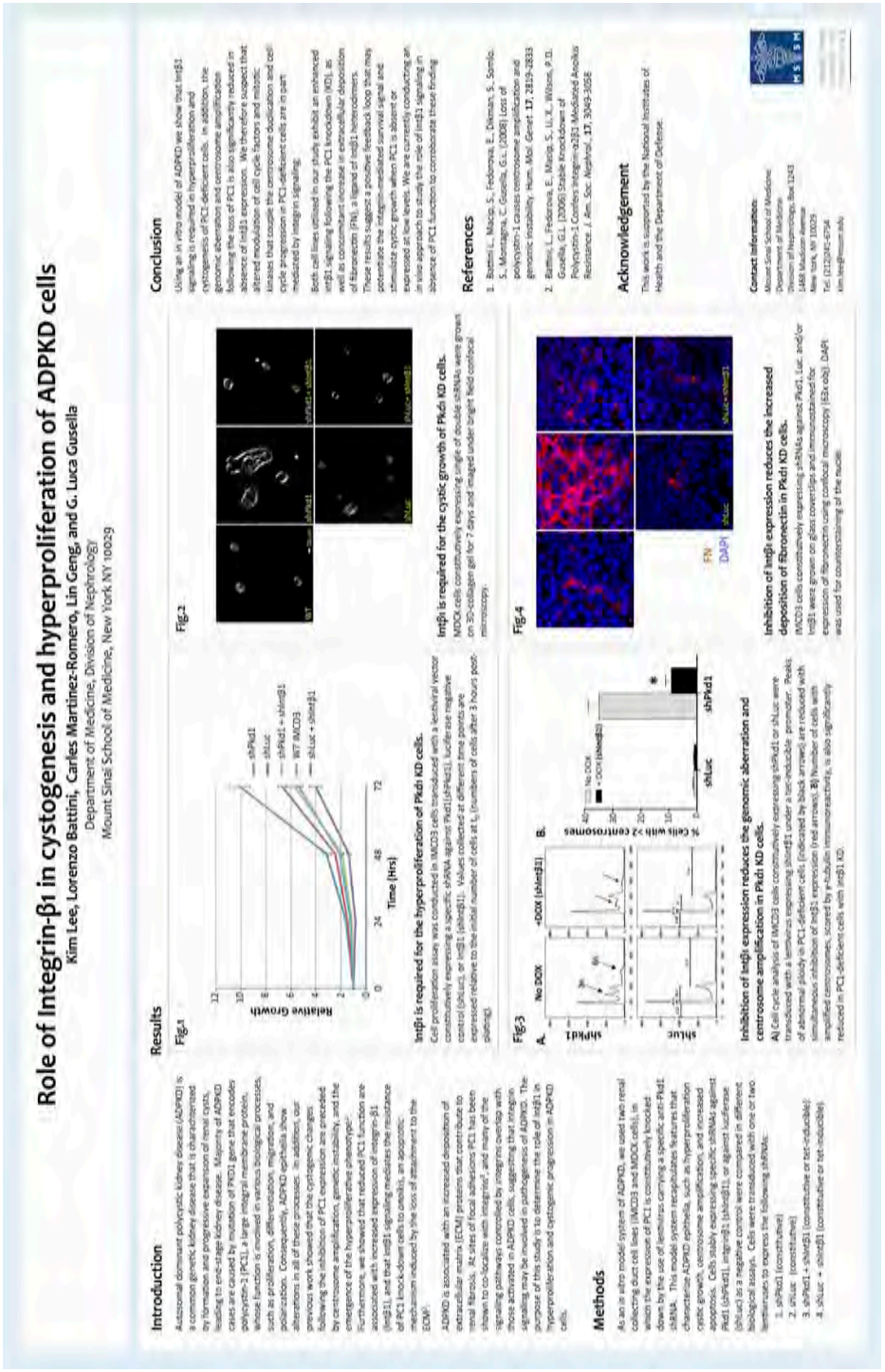
## REFERENCES


1. Battini L., Macip S., Fedorova E., Dikman S., Somlo S., Montagna C., Gusella G.L. Loss of polycystin-1 causes centrosome amplification and genomic instability. *Human molecular genetics* 17:2819-2833 (2008).
2. Zhang J., Wu M., Wang S., Shah J., Wilson P.D., Zhou J. Polycystic kidney disease protein fibrocystin localizes to the mitotic spindle and regulates spindle bipolarity. *Human molecular genetics* (2010).
3. Tammachote R.*, et al.* Ciliary and centrosomal defects associated with mutation and depletion of the Meckel syndrome genes MKS1 and MKS3. *Human molecular genetics* 18:3311-3323 (2009).
4. Battini L., Fedorova E., Macip S., Li X., Wilson P.D., Gusella G.L. Stable knockdown of polycystin-1 confers integrin-α2β1-mediated anoikis resistance. *J Am Soc Nephrol* 17:3049-3058 (2006).
5. Piontek K.B.*, et al.* A functional floxed allele of Pkd1 that can be conditionally inactivated in vivo. *J Am Soc Nephrol* 15:3035-3043 (2004).
6. Zhang X.*, et al.* beta1 integrin is necessary for ureteric bud branching morphogenesis and maintenance of collecting duct structural integrity. *Development* 136:3357-3366 (2009).

7. Raphael K., Strait K., Stricklett P., Miller R., Nelson R., Piontek K., Germino G., Kohan D. Inactivation of Pkd1 in principal cells causes a more severe cystic kidney disease than in intercalated cells. *Kidney Int* 75:626-633 (2009).
8. Lu W.*, et al.* Comparison of Pkd1-targeted mutants reveals that loss of polycystin-1 causes cystogenesis and bone defects. *Hum Mol Genet* 10:2385-2396 (2001).

## APPENDICES

Appendix 1



# Uromodulin Controls Centrosome Integrity in Cells of the Thick Ascending Limb of the Loop of Henle

Lorenzo Battini, *[1], Kim Lee[1], Carles Martinez-Romero[1], Lin Geng[1] and G. Luca Gusella[1].
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## ABSTRACT

Mutations of the *uromodulin* gene, *UMOD*, are responsible for familial juvenile hyperuricemic nephropathy (FJHN) and medullary cystic kidney disease type 2 (MCKD2). Both diseases are autosomal dominant tubulointerstitial nephropathies, characterized by hyperuricemia, gout, hypertension, cysts formation, and end-stage renal disease. In spite of the fact that uromodulin is the most abundant protein present in the urine, its biological function is not completely understood. We have created a novel cell line, TIRE (Telomerase-Immortalized Renal Epithelial cells), by infecting human primary epithelial cells with a lentiviral vector overexpressing human telomerase. Characterization of a specific clone, TIRE 13-1, showed characteristic morphological and functional properties of the thick ascending limb of the loop of Henle. To date, this is the only human cell line with this specific characteristic and therefore represents a valuable tool to study UMOD-associated diseases.
The siRNA lentiviral-mediated knock-down of UMOD showed centrosome amplification and genomic instability in TIRE 13-1 cells, but not in MDCK or IMCD cells. Interestingly, the knock-down of PKD1 had the opposite effects, causing centrosomes amplification in MDCK and IMCD cells but not in TIRE13-1. It has been well established that in ADPKD patients cysts seem to be derived largely from collecting ducts and distal tubules, where polycystin-1 is mostly abundant in the adult kidney, whereas in UMOD-related diseases, cysts arise from the medulla or at the corticomedullary junction. These results raise the intriguing possibility that in different segment of the nephron in the adult kidney the cytoarchitecture is controlled by different genes.

## MATERIALS AND METHODS

**Cell cultures and reagents**
Cell lines were maintained at 37°C in 5% $CO_2$ in DMEM supplemented with 10% FBS (MDCK cells) or in a 1:1 (vol:vol) of DMEM/F12 medium supplemented with 10% FBS (Telomerase Immortalized Renal Epithelial cells), TIRE13-1, and the transduced cell lines: TIREsiUMOD86, TIREsiUMOD17 and TIREsiLuc.

**Immunodetection**
Cells were harvested by scraping, washed twice in PBS and lysed in RIPA buffer containing Complete Protease and Phosphatase Inhibitors Cocktail. Protein extracts (100 µg) from each sample were resolved by SDS-polyacrylamide gel electrophoresis on a 4–12% gradient gel and electrophoretically blotted onto immobilon-P membrane, and incubated with primary rabbit polyclonal anti-Uromodulin or rabbit polyclonal anti-Aquaporin2 (H135, or H40, Santa Cruz Biotechnology, Santa Cruz, CA) in 5% milk in PBS containing 0.05% Tween-20. Immunocomplexes were detected with peroxidase-conjugated goat anti-rabbit IgG antibody (1:5000) and the enhanced chemiluminescence detection kit, Lumi-Light Plus.

**Cell cycle analysis**
At the indicated time points post-transduction, cells were washed twice with PBS, trypsinized and resuspended at $10^6$ cells/ml in DMEM/F2 supplemented with 10% FCS. The samples were then processed using the CycleTEST PLUS following the manufacturer's protocol, and run through a FACSCan flow cytometer.Data were analyzed using the FlowJo software.

**Immunohistochemistry**
Cells grown on glass coverslips were fixed with −20°C methanol for 10 min, and then blocked in 10%NDS for 60 min at room temperature. Samples were then incubated with the indicated primary antibodies in PBS 3% BSA for 60 min and then washed three times for 5 min with PBS and the incubated for 30 minutes with fluorescent antibodies in the dark. The slides were then mounted in VectashNd anti-fade reagent with DAPI and analyzed with a Leica confocal fluorescence microscope.

**Three-Dimensional Collagen Gels**
TIRE13-1 and stably transduced cells: TIREsiUMOD17 and TIREsiLuc. were trypsinized to a single-cell suspension and resuspended at a concentration of $1^* 10^5$ cells/ml in an ice-cold collagen solution, briefly, collagen type I solution was mixed with 10X DMEM and 7.5% (vol/vol) Bicarbonate solution in a ratio of 8:1:1. The cell suspension then was dispensed in droplets of 25µl in six-well plates and allowed to gel for 20 min at 37°C before being overlaid with 1.5 ml DMEM/F12 medium that was changed every 3 days, and pictures were taken at day 14.

## TIRE13-1 cells are derived form the thick ascending limb of the loop of Henle

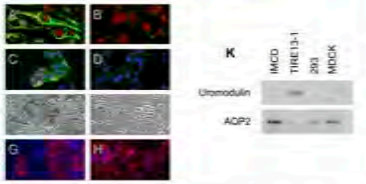


Fig.1 Caracterization of TIRE13-1 cells. Immunofluorescence staining with anti-Cytokeratin-8 Ab (green) **A**, anti-Cytokeratin-19 Ab (green) **B**, anti-Aquaporin-2Ab (green) / **D**, Alkaline Phosphatase **F**, anti-Uromodulin Ab (red) **H**, or control cell lines, MDCK with anti-Aquaporin-2 Ab (green) **C**, LLCPK with Alkaline Phosphatase **E**, and Medullary section of a mouse kidney with anti-Uromodulin Ab (red) **G**. Immunoblotting of IMCD, TIRE13-1, 293, and MDCK cells **K**, with anti-Uromodulin (top panel)or anti-Aquaporin-2 Ab (lower panel)(AQP2).

## Uromodulin knockdown causes centrosome amplification, genomic instability, and impairs the process of tubulogenesis in TIRE13-1 cells

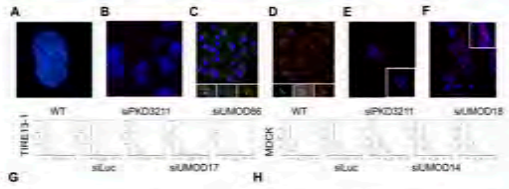


Fig.3 Immunofluorescence staining five days post transduction of Uromodulin or control Luciferase knockdown Tire13-1 cells with: centrosomal protein γ Tubulin (red), **A, B**, and Acetilated Tubulin (green), Uromodulin (red), **C, D**. Immunostaining of siUMOD86 or control siLuc knockdown Tire13-1 cells in three-dimensional collagen gels, after 14 days of culture, with Phalloidin anti-actin (red), **E, F**. Cell cycle analisys of Tire13-1 or MDCK cells, infected with different siRNA five days post transduction, **G, H**.

## TIRE13-1 conserve their original phenotypical and physiological characteristics

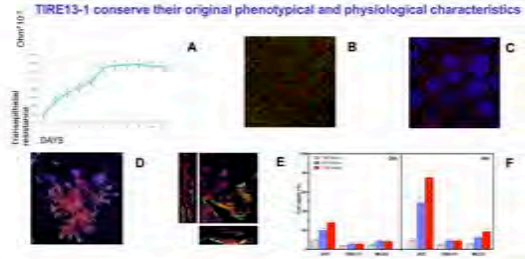


Fig.2 Polarization curve of Tire-13-1 cells after 12 day of been cultured at confluency on a transwell. **A** (error bars represent an average of 3 independent experiments). Immunofluorescence staining of TIRE13-1 cells cultured on transwell with: anti-ZO1 Ab (green) **B**, or anti-AcetilatedTubulin Ab (red) **C**. Immunofluorescence staining of TIRE13-1 tubules formed in three-dimensional collagen gels with: anti-AcetilatedTubulin (green)Ab, Phalloidin anti-Actin (red) and DAPI counterstaining, (10X) Confocal microscopy **D**, or (60X) Confocal microscopy **E**. Hyperosmotic Resistance **F**, of 293, Tire13-1, or IMCD cells performed at 24 and 48h with 3 different Osmolarity.

## CONCLUSIONS

- TIRE13-1 are a unique human renal epithelial cell line with phenotypic and physiological characteristics of the thick ascending limb of the loop of Henle
- TIRE13-1 undergo spontaneous tubulogenesis when grown in 3D collagen gels, and may be useful as a novel *in vitro* model for the study the cystogenic process of uromodulin-related diseases.
- Knockdown of UMOD resulted in centrosome amplification and genomic instability in TIRE 13-1 cells, but not in MDCK. Conversely, PKD1 knockdown causes centrosome amplification in MDCK cells but not in TIRE13-1.

Overall, these results suggest that centrosome anomalies and genetic instability may be a common event of the cystogenic transformation, regardless of the causative cystic gene. Furthermore, they raise the intriguing possibility that different genes control the cytoarchitecture of different segments of the nephron.

Appendix 3

# Primary cilia dynamics instruct tissue patterning and repair of corneal endothelium

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Edited* by Kathryn V. Anderson, Sloan-Kettering Institute, New York, NY, and approved January 11, 2011 (received for review November 10, 2010)

**Primary cilia are required for several signaling pathways, but their function in cellular morphogenesis is poorly understood. Here we show that emergence of an hexagonal cellular pattern during development of the corneal endothelium (CE), a monolayer of neural crest-derived cells that maintains corneal transparency, depends on a precise temporal control of assembly of primary cilia that subsequently disassemble in adult corneal endothelial cells (CECs). However, cilia reassembly occurs rapidly in response to an in vivo mechanical injury and precedes basal body polarization and cellular elongation in mature CECs neighboring the wound. In contrast, CE from hypomorphic *IFT88* mutants (*Tg737*$^{orpk}$) or following in vivo lentiviral-mediated IFT88 knockdown display dysfunctional cilia and show disorganized patterning, mislocalization of junctional markers, and accumulation of cytoplasmic acetylated tubulin. Our results indicate an active role of cilia in orchestrating coordinated morphogenesis of CECs during development and repair and define the murine CE as a powerful in vivo system to study ciliary-based cellular dynamics.**

intraflagellar transport | eye development | ciliary length | microtubules

During development and tissue repair, groups of cells achieve or reconstitute highly organized patterns to form complex tissues by precisely integrating extracellular cues. How each cell converts such cues into events of cellular morphogenesis in a timely and coordinated manner is not understood (1). Primary cilia are microtubule-based antenna-like organelles protruding from the surface of vertebrate cells that mediate a number of signaling pathways during development and tissue homeostasis (2–4). The assembly, maintenance, and disassembly of cilia depend on intraflagellar transport (IFT) (5, 6). Null mutations affecting components of the IFT lead to patterning defects and midgestation lethality of the mouse embryo (7, 8). Cilia malfunction due to abnormal composition or function results in a spectrum of human syndromes referred to as ciliopathies (9–11).

As the cilium is reabsorbed before cell division, it is generally assumed that cilia appear in cells that have exited the cell cycle and entered quiescence or differentiation (12). Whereas this is true for many adult tissues, in some epithelial cells, such as the hair cells in the cochlea and the luminal cells of the mammary gland, the primary cilium regresses in the adult stage (13, 14). This led us to hypothesize that the resorption of the cilium may represent, in specific cell types, a signal of complete maturation and achievement of the normal adult cell shape and configuration in a tissue. Therefore, by controlling the assembly and disassembly of their primary cilium, cells could modulate the response to extracellular morphogenetic signals and determine their cellular architecture. Given the tridimensional nature of complex tissues, often comprising multiple cellular layers of different embryological origins, it has been difficult to follow the dynamics of ciliary assembly and disassembly in specific groups of cells and determine how these events correlate with cellular morphogenesis during development or tissue repair.

The corneal endothelium (CE), which plays an essential role in maintaining corneal transparency, offers the advantage of being organized in a 2D monolayer of hexagonal cells facing the anterior chamber of the vertebrate eye (15, 16). Because of its accessible location and simple well-defined organization, this tissue constitutes a valuable model system to study cellular morphogenesis in the context of a complex tissue during development and repair.

Although the patterning and cell density of this tissue are critical in maintaining the normal physiology of CECs, the developmental steps that control these processes remain largely unknown. Here we provide evidence that primary cilia of the mouse corneal endothelium are required to allow proper morphogenesis toward the characteristic hexagonal cell patterning of the CE during postnatal development. Furthermore, we demonstrate that primary cilia assemble only when corneal endothelial cells undergo morphogenesis during development and tissue repair but disassemble during tissue homeostasis in adult animals.

## Results and Discussion

**Primary Cilia Transiently Assemble on CECs from the Periphery to the Center of the CE.** Several studies describe a primary cilium of unknown function on CECs of different vertebrates; however, there are discrepancies about whether all or only a subpopulation of adult CECs are ciliated (17–19). Because CECs undergo profound age-dependent morphological changes, we asked whether the presence or absence of primary cilia is associated with a specific developmental stage (or age) (20). We analyzed the CE of mice from E17.5 to adulthood. Primary cilia and basal bodies were identified by immunofluorescence using a monoclonal antibody to acetylated-α-tubulin (21), a major component of the primary cilium, and a polyclonal antibody to γ-tubulin, respectively. Cell–cell contacts were visualized with a polyclonal antibody against the tight junction protein ZO-1. Only structures stemming from basal bodies were counted as primary cilia. Primary cilia were undetectable in CE of E18.5 embryos (Fig. S1). They started to assemble as early as postnatal day 1 (1 d) but were absent in adult animals (>45–75 d; Fig. 1) In 1- to 2-d-old mice, primary cilia of about 2–3.5 μm were predominantly present on CECs located at the periphery of the cornea (Fig. 1*B*), whereas CECs present at the center of the CE only displayed a very short cilium (Fig. 1*A*). By 4 d, cilia of CECs at the center also reached a length of about 2–3 μm that persisted through 12 d, when most of the peripheral cilia began to shorten (Fig. 1 *C* and *D*). By 33–35 d, the majority of peripheral and central CECs had lost their cilia. The remaining ciliated cells presented a cilium no longer than 2 μm and were more frequent at the center than at the periphery of CE (Fig. 1 *E* and *F*). The CE of a 70-d mouse had virtually no cilia (Fig. 1*G*). Fig. 1*H* summarizes the dynamic changes of cilia length at the center and periphery of the CE at various postnatal stages.

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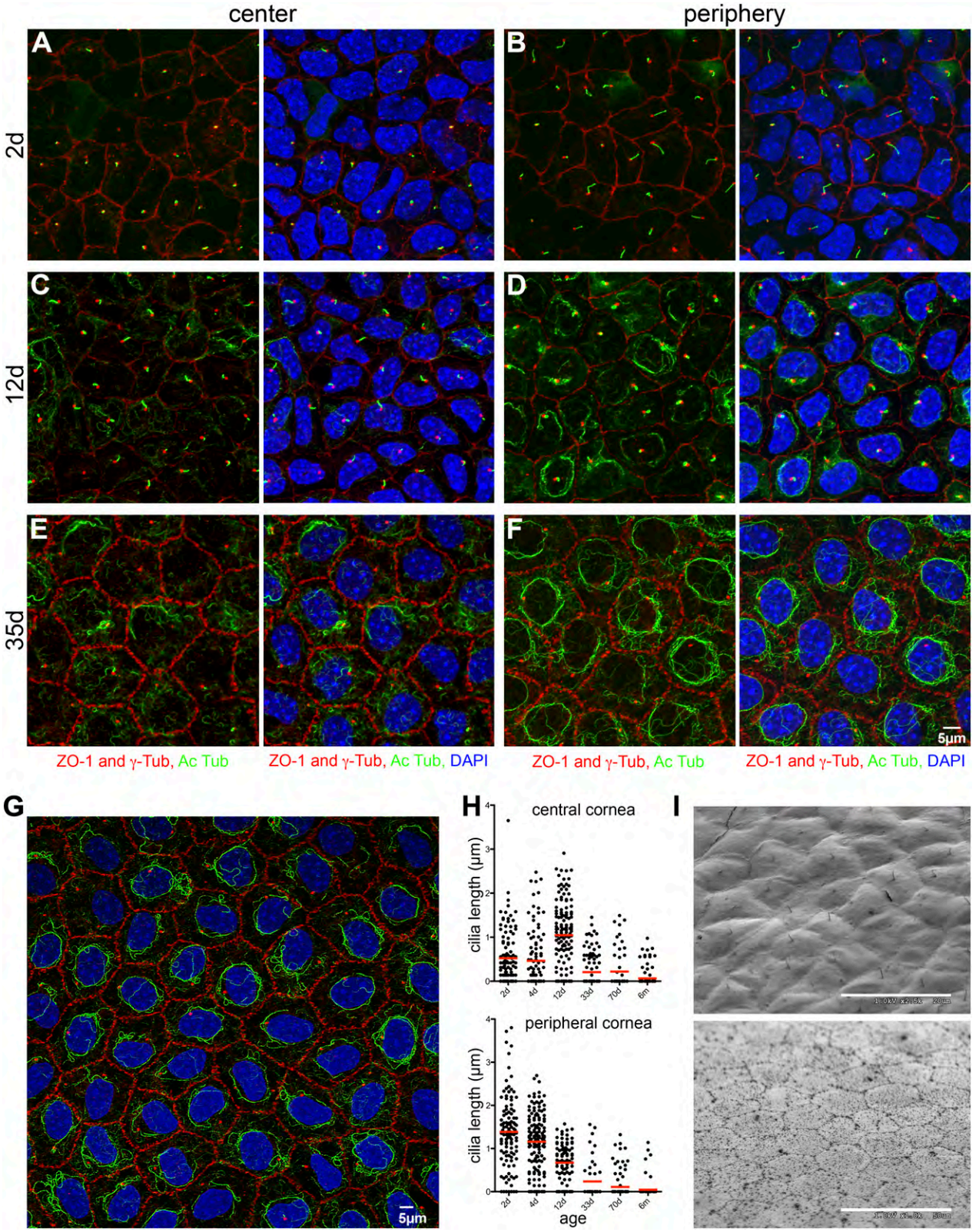


**Fig. 1.** Primary cilia assembly and disassembly are dynamically regulated during cellular morphogenesis and development of CE. (*A–G*) Confocal immunomicroscopy of cornea flat mount from mice at different postnatal developmental stages showing the peripheral or central areas of CE. ZO-1Ab stains cell–cell junctions and γ-tubulin the basal body at the cell center; both are shown in red, acetylated tubulin in green, and nuclei in blue. (*H*) Distribution of cilia length at the center and periphery of the cornea. On average ~100 cells at the center or at the periphery of the same cornea were counted for each age group. A similar distribution of cilia length was obtained when at least two additional corneas for each age group were analyzed. (*Y* = % and *X* = age. (*I*) SEM of CE from mice of 9 d and 7 mo reveals the presence and the absence of cilia, respectively.

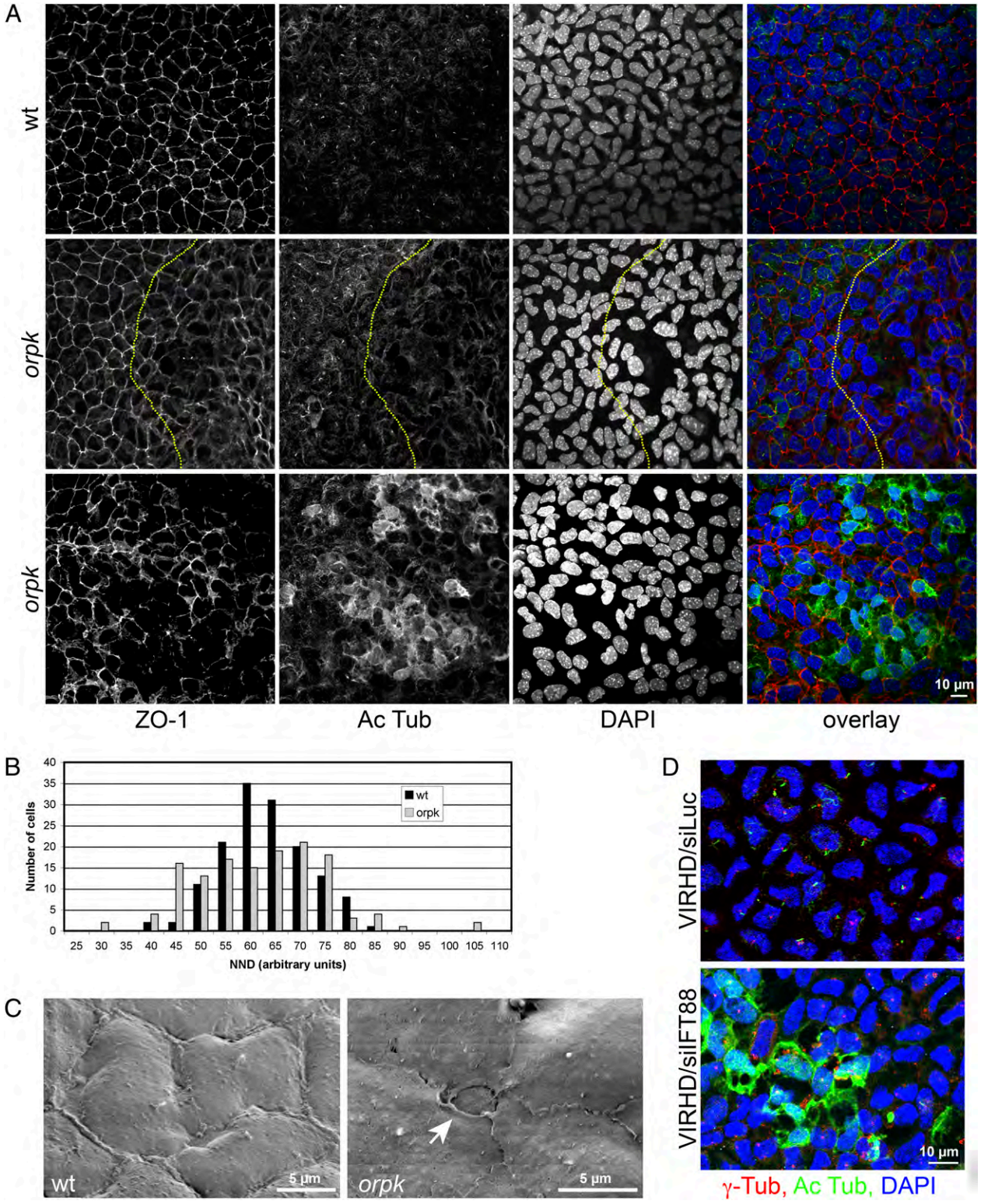


**Fig. 2.** Ciliary dysfunction leads to defects in CE patterning. (*A*) Confocal immunomicroscopy of 6-d-old *Tg737*$^{orpk}$ and wild-type CE flat mount stained with indicated antibodies. (*Middle*) Only areas lacking cilia (*Right* of the dotted line) are defective in nuclei and ZO-1 distribution. (*Bottom*) Defect seems more severe and in addition to the "packaging" phenotype there is also a remarkable accumulation of cytoplasmic acetylated tubulin. (*B*) NND analysis for each nucleus was obtained by comparing the thresholded image of the nuclei in *A*, *Upper* and *Lower*). (*C*) SEM of 5-d-old wild-type and *Tg737*$^{orpk}$ CE, *Upper* and *Lower*, respectively, Note the defect in cell–cell contact formation indicated by the arrow and blunted cilia. (*D*) Immunofluorescence of CE from 15-d-old mice injected with a lentivirus expressing the siRNA against IFT88 and a control lentivirus expressing the siRNA against luciferase. Mice were injected 6 d after birth and killed at age 15 d; the experiment was repeated twice with similar outcome. IFT88 knockdown phenocopies *Tg737*$^{orpk}$.

Our data suggest that assembly of primary cilia in the CE is temporally regulated, starting perinatally first on peripheral CECs and then proceeding gradually to centrally located CECs in a wave-like manner. Likewise, cilia progressively disassemble in CECs of mice older than 15 d starting from the periphery toward the center of the CE until almost complete absence in 45-d-old mice. Cilia disassembly was confirmed by analyzing CE at different postnatal time points by scanning electron microscopy (SEM) (Fig. 1*I* and Fig. S2). Interestingly, resorption of cilia coincided precisely with the progressive formation of a hexagonal CEC pattern. Early after birth, CECs appear as irregular polygons with a variable number of sides and no detectable acetylated microtubules in the cytoplasm. By day 30–35, CECs achieved a hexagonal shape with characteristic bundles of acetylated microtubules around the nucleus (Fig. 1 *A*–*F*).

**CE Cellular Pattern Is Altered in *Tg737*$^{orpk}$ Mice, a Hypomorphic Allele of IFT88.** The tight correlation between shape changes, cytoskeletal remodeling, and cilia assembly and disassembly in CECs during postnatal development led us to hypothesize that the cilium of CECs controls CE patterning. Null mutations that completely abolish cilia lead to midgestation lethality, before the CE layer is established (22). The IFT88 protein is a component of the IFT, the bidirectional movement of particles between the tip and the base of the cilium required for cilia assembly and maintenance first described in *Chlamydomonas* (23). The hypomorphic *Ift88* allele in the *Tg737*$^{orpk}$ mouse leads to cilia that are stunted and malformed, but not completely abolished, thus allowing these homozygous mutants to survive within the weaning period (24). In contrast, mice that are heterozygous for this mutation appear indistinguishable from wild-type littermates (25). To determine CE patterning in tissues with aberrant cilia, we isolated corneas from *Tg737*$^{orpk}$ mice ($n = 5$) and wild-type or heterozygous littermates at different postnatal developmental ages between 4 d and 19 d. To visualize the cell contacts, primary cilia, and nuclei of CECs, flat mounted corneas were stained with antibodies against ZO-1, acetylated tubulin, and DAPI. Cilia length and distribution differed significantly between *Tg737*$^{orpk}$ homozygotes compared with control littermates. Whereas some areas of CE in the corneas of *Tg737*$^{orpk}$ homozygotes exhibited cilia of fairly normal length, other areas presented short or absent cilia (Fig. 2*A*, *Middle* and *Bottom*, respectively). Within CE areas with defective cilia, CECs displayed a disorganized cellular pattern, ZO-1 mislocalization, and cytoplasmic accumulation of acetylated tubulin. SEM micrographs occasionally showed incomplete cellular junction formation between CECs with short cilia in *Tg737*$^{orpk}$ mice (Fig. 2*C*). In contrast, all CECs in wild-type or heterozygous littermates showed normal cilia and cellular distribution (Fig. 2*A*, *Top*). The patterning of the CECs in mutant mice was compared with that in wild-type using an image analysis approach and quantified (Fig. 2*B*). Notice that the nearest neighbor distance (NND) distribution of the CEC nuclei in *Tg737*$^{orpk}$ was much wider than that of wild-type controls. To determine whether the aberrant spacing of the nuclei in the CE of *Tg737*$^{orpk}$ was a consequence of cell death we performed TUNEL analysis on developing CE. No TUNEL-positive cells were detected on CE of 4-d *Tg737*$^{orpk}$ and wild-type littermate mice (Fig. S3). In addition, the CEC density is similar in both wild-type and mutant strains as assessed in 6-d old mice [wild type, 154 cells/0.02 mm$^2$ ($n = 3$) and *Tg737*$^{orpk}$, 151.3 cells/0.02 mm$^2$ ($n = 3$)]. These results suggest that apoptosis does not account for CE patterning defects detected in the *Tg737*$^{orpk}$ mouse. Next, we tested whether the abnormal tissue patterning in *Tg737*$^{orpk}$ CE is due to defective morphology of individual CECs. We measured the area and the "shape factor" of the polygons identified by the cell–cell contacts in the CE labeled by the ZO-1 antibody in both strains. The distribution of the polygonal areas in the CE of 6-d *Tg737*$^{orpk}$ mice was wider than that of wild-type littermates and the means of the CEC shape factor was 0.64 and 0.71 in the *Tg737*$^{orpk}$ and wild-type mice, respectively, implying an intrinsic defect of the overall architecture of the CECs in the *Tg737*$^{orpk}$ mice (Fig. S4). Taken together, these results suggest a critical role of IFT88 in the cytoskeletal remodeling and cellular morphogenesis of CECs required for tissue patterning in the developing CE.

Given the nonconditional nature of the *Tg737*$^{orpk}$ mutation, we cannot however exclude that the CE phenotype of newborn *Tg737*$^{orpk}$ mice resulted from defective interactions between neural crest progenitors of CECs and other ciliated cells early in development or from a role of IFT88 in neural crest cell migration similar to that described for other basal body and ciliary proteins (26). To exclude these possibilities, we knocked down IFT88 expression in CECs in vivo using a lentiviral-mediated RNAi approach.

**Lentiviral-Mediated IFT88RNAi Phenocopies *Tg737*$^{orpk}$ Defects in CE.** As previously shown, primary cilia persist on mouse CECs for about 4 wk after birth. During this time, CECs undergo morphogenesis to acquire a hexagonal shape (Fig. 1). To specifically inactivate IFT88 in the CE, we injected the anterior chamber of 5- to 6-d old mice with 2 μL of VIRHD/E/siLuc or VIRHD/E/siIFT88 lentivectors expressing an siRNA hairpin against luciferase (control) or the murine IFT88 gene, respectively (27). Transduction rates in vivo and cell-type specificity were monitored by injecting the VVPW/H2B-cherry lentivirus expressing cherry-tagged histone H2B under the constitutive CMV promoter. Virtually all CECs, but no other corneal cell types, expressed H2B-cherry in the nucleus (Fig. S5).

Corneas were isolated 5–7 d after injection and analyzed by immunofluorescence. Several areas of the CE from eyes injected with VIRHD/E/siIFT88 showed defects similar to those affecting the CE of *Tg737*$^{orpk}$ mice: absent or shortened cilia and corresponding abnormal patterning of CECs (Fig. 2*D*). In contrast, none of these defects were observed in CEs of mice injected with the control lentivector VIRHD/E/siLuc. These results indicate that primary cilia play an instructive role in determining the normal patterning of the CE during postnatal development.

**Primary Cilia Assemble During in Vivo CE Repair in Adult Mice.** We next asked whether components of the IFT machinery that are required for ciliary assembly were still present in CECs of adult mice. Staining with antibodies specific for IFT72/74 (also known as CMG-1Ab) (28) and IFT88 was detected along the cilium and at the basal body of CECs in 4-d-old mice and at the basal body of CECs in 7-mo-old mice (Fig. 3 and Fig. S6). Therefore, IFT components persist at the basal body of CECs in adult mice,

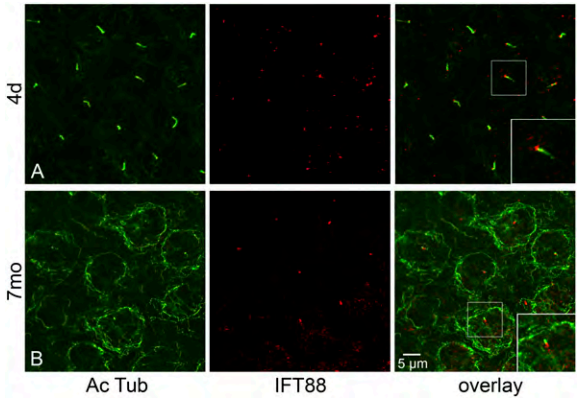


**Fig. 3.** IFT proteins in CE of young and adult mice. Immunofluorescence of flat mount of CE from 4-d- and 7-mo-old mouse stained with acetylated tubulin Ab (green) and IFT88Ab (red).

suggesting that CECs maintain the ability to reassemble a primary cilium.

To further address the notion that cilia orchestrate cellular morphogenesis of CECs, we tested the hypothesis that the cilium reassembles when cellular morphogenesis is reinduced in the CE of adult mice by wounding. As CECs show little or no proliferation during wound repair, they stretch and migrate in a direction perpendicular to the wound to cover the exposed Descemet's membrane, losing their normal hexagonal shape (29). Typically, after 48 h, a wound of about 3 mm is repaired by a complete layer of enlarged CECs, characterized by an irregular shape, which persists for several months (30). To investigate a possible role of primary cilia in endothelial repair in vivo, we produced a linear endothelial wound in the CE of adult mice. The wound was generated by gently scraping cells with a 10.0 suture surgical needle inserted into the anterior chamber of the eye of 5- to 6-mo-old mice and passed across the CE. After 18 h and 30 h of healing time, mice were killed and corneas were removed and analyzed. Cilia of about 2–3 μm were detected on CECs near the wound (4–5 cell rows) as early as 18 h after wounding (Fig. 4*A*). Although newly ciliated CECs within 4–5 cell rows next to the wound maintained a shape close to a hexagon, the perinuclear bundles of acetylated microtubules were disorganized or absent and the basal bodies localized at the median of the cell (Fig. 4 *A* and *D*). In corneas isolated 30-h postwounding, CECs neighboring the wound still showed a primary cilium and lacked cytoplasmic acetylated microtubules. In addition, their shape was elongated in the axis perpendicular to the wound and the basal bodies polarized to the cell side closer to the wound (Fig. 4 *B* and *D*). In contrast, CECs away from the

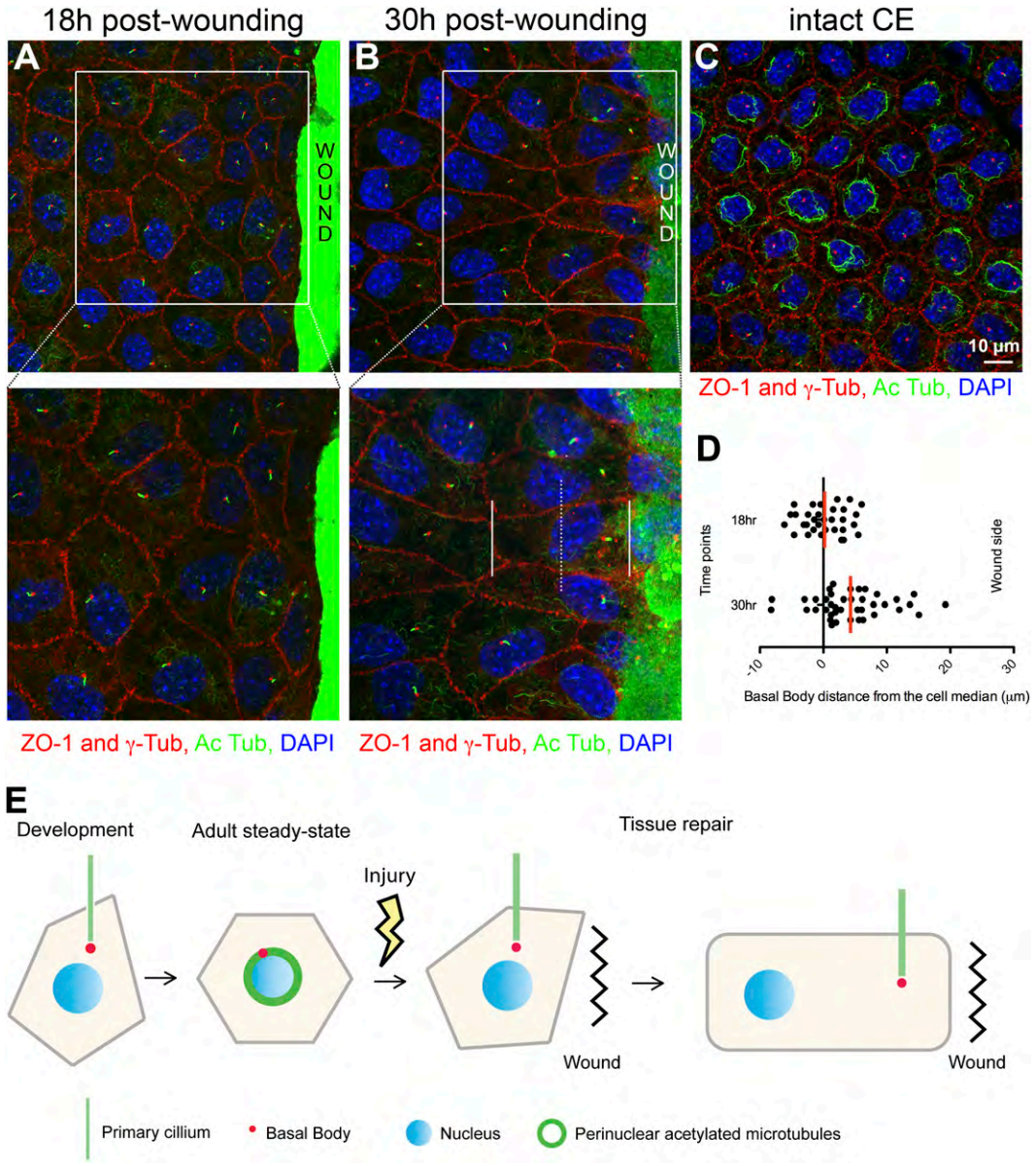


**Fig. 4.** Cilia assembly, cellular remodeling, and basal body polarization of CECs during CE repair. (*A* and *B*) Wounded area of CE 18 h and 30 h after in vivo recovery. Corneas were dissected from 3-mo-old mice. (*C*) Intact area of the same CE shown in *B*, located ~150–200 cell rows away from the wounded area. (*D*) Basal body distance from the cell median, measured as shown by the dotted line in *B*, *Bottom*. (*E*) Model of the cilia dynamics during cellular morphogenesis in development and repair (see text). Acetylated tubulin is shown in green, ZO-1 and γ-tubulin in red, DAPI in blue. Data corroborating the reorientation of the basal body were obtained in additional independent experiments and are shown in Fig. S7.

wounded area (~150–200 cell rows) remained aciliated and maintained their hexagonal shape, the perinuclear bundle of acetylated microtubules, and the basal bodies localized near the cell center (Fig. 4*C*).

Taken together, our data indicate that cilia are present on CECs during events of cellular remodeling, occurring in development or tissue repair of the CE, but disassemble during steady state in normal adult CE (Fig. 4*E*). In support of this idea, we have shown that ciliary dysfunction leads to defective patterning of CE during postnatal development. Intriguingly, findings in other tissues support similar conclusions. The kinocilium, the primary cilium found in inner ear hair cells, is required for proper development of the organ of Corti but it is reabsorbed about 10 d after birth. However, following trauma, hair cells from differentiated cochlea reassemble their kinocilia regardless of age, as shown in in vivo and ex vivo experiments in mice and rabbits (31, 32). During development and repair, inner ear hair cells undergo morphogenesis and show intense remodeling of the microtubular and actin cytoskeleton, and the kinocilium controls the localization of the basal bodies within hair cells (31, 33). In addition, primary cilia in adult kidney cells not only act as flow sensors, but may also be involved in epithelial differentiation during renal injury and repair. For example in mice, tubular damage causes an increase and a subsequent regression of cilium length during renal repair (34). Moreover *foxj1a*, the transcriptional regulator of cilia genes, is rapidly induced in response to epithelial stretch or injury (35). Thus, in general, the reassembly of primary cilia in adult tissues seems to represent a very early response to an injury. Interestingly, *Tg737*$^{orpk}$ mice show defects in wound closure (36). Recent studies have shown that spatial constraint and actin cytoskeleton remodeling can control cilia elongation and resorption (37). It is tempting to speculate that mechanical forces generated during tissue patterning and disrupted by an injury can modulate the cellular response to cilia-mediated signaling by controlling primary cilia dynamics. Identifying the cues and the mechanism promoting ciliary assembly upon tissue damage, and the signaling pathways mediated by this organelle during the healing process, could improve our understanding of how to treat injuries. Due to its simple organization as a monolayer facing the ocular anterior chamber and its accessibility to lentivector-mediated gene transfer that can be monitored in vivo by confocal microscopy, the CE provides a unique valuable model system to study the dynamic properties and function(s) of primary cilia in cellular morphogenesis during tissue development and repair.

## Materials and Methods

The mouse strains and oligonucleotide sequences used in this study as well as a detailed description of lentiviral production, microscopy and morphometric analysis, and in vivo wound healing experiments are provided in *SI Materials and Methods*.

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1. Davies JA (2005) *Mechanisms of Morphogenesis* (Academic, London).
2. Berbari NF, O'Connor AK, Haycraft CJ, Yoder BK (2009) The primary cilium as a complex signaling center. *Curr Biol* 19:R526–R535.
3. Christensen ST, Pedersen LB, Schneider L, Satir P (2007) Sensory cilia and integration of signal transduction in human health and disease. *Traffic* 8:97–109.
4. Pazour GJ, Witman GB (2003) The vertebrate primary cilium is a sensory organelle. *Curr Opin Cell Biol* 15:105–110.
5. Pan J, Snell WJ (2005) Chlamydomonas shortens its flagella by activating axonemal disassembly, stimulating IFT particle trafficking, and blocking anterograde cargo loading. *Dev Cell* 9:431–438.
6. Rosenbaum JL, Witman GB (2002) Intraflagellar transport. *Nat Rev Mol Cell Biol* 3: 813–825.
7. Sharma N, Berbari NF, Yoder BK (2008) Ciliary dysfunction in developmental abnormalities and diseases. *Curr Top Dev Biol* 85:371–427.
8. Goetz SC, Anderson KV (2010) The primary cilium: A signalling centre during vertebrate development. *Nat Rev Genet* 11:331–344.
9. Badano JL, Mitsuma N, Beales PL, Katsanis N (2006) The ciliopathies: An emerging class of human genetic disorders. *Annu Rev Genomics Hum Genet* 7:125–148.
10. Marshall WF (2008) The cell biological basis of ciliary disease. *J Cell Biol* 180: 17–21.
11. Zariwala MA, Knowles MR, Omran H (2007) Genetic defects in ciliary structure and function. *Annu Rev Physiol* 69:423–450.
12. Veland IR, Awan A, Pedersen LB, Yoder BK, Christensen ST (2009) Primary cilia and signaling pathways in mammalian development, health and disease. *Nephron, Physiol* 111:39–53.
13. Kikuchi K, Hilding D (1965) The development of the organ of Corti in the mouse. *Acta Otolaryngol* 60:207–222.
14. McDermott KM, Liu BY, Tlsty TD, Pazour GJ (2010) Primary cilia regulate branching morphogenesis during mammary gland development. *Curr Biol* 20:731–737.
15. Cvekl A, Tamm ER (2004) Anterior eye development and ocular mesenchyme: New insights from mouse models and human diseases. *Bioessays* 26:374–386.
16. Hogan MJ, Alvarado JA, Weddell JE (1971) *Histology of the Human Eye* (Saunders, Philadelphia).
17. Doughty MJ (2004) Influence of initial fixation protocol on the appearance of primary cilia on the rabbit corneal endothelial cell apical surface as assessed by scanning electron microscopy. *Cell Biol Int* 28:131–137.
18. Collin SP, Collin HB (1998) A comparative study of the corneal endothelium in vertebrates. *Clin Exp Optom* 81:245–254.
19. Svedbergh B, Bill A (1972) Scanning electron microscopic studies of the corneal endothelium in man and monkeys. *Acta Ophthalmol (Copenh)* 50:321–336.
20. Murphy C, Alvarado J, Juster R, Maglio M (1984) Prenatal and postnatal cellularity of the human corneal endothelium. A quantitative histologic study. *Invest Ophthalmol Vis Sci* 25:312–322.
21. Piperno G, LeDizet M, Chang XJ (1987) Microtubules containing acetylated alpha-tubulin in mammalian cells in culture. *J Cell Biol* 104:289–302.
22. Saika S, et al. (2001) TGFbeta2 in corneal morphogenesis during mouse embryonic development. *Dev Biol* 240:419–432.
23. Pazour GJ, et al. (2000) *Chlamydomonas IFT88* and its mouse homologue, polycystic kidney disease gene *tg737*, are required for assembly of cilia and flagella. *J Cell Biol* 151:709–718.
24. Lehman JM, et al. (2008) The Oak Ridge Polycystic Kidney mouse: Modeling ciliopathies of mice and men. *Dev Dyn* 237:1960–1971.
25. Moyer JH, et al. (1994) Candidate gene associated with a mutation causing recessive polycystic kidney disease in mice. *Science* 264:1329–1333.
26. Tobin JL, et al. (2008) Inhibition of neural crest migration underlies craniofacial dysmorphology and Hirschsprung's disease in Bardet-Biedl syndrome. *Proc Natl Acad Sci USA* 105:6714–6719.
27. Battini L, et al. (2006) Stable knockdown of polycystin-1 confers integrin-alpha2beta1-mediated anoikis resistance. *J Am Soc Nephrol* 17:3049–3058.
28. Iomini C, Tejada K, Mo W, Vaananen H, Piperno G (2004) Primary cilia of human endothelial cells disassemble under laminar shear stress. *J Cell Biol* 164:811–817.
29. Joyce NC (2003) Proliferative capacity of the corneal endothelium. *Prog Retin Eye Res* 22:359–389.
30. Tuft SJ, Williams KA, Coster DJ (1986) Endothelial repair in the rat cornea. *Invest Ophthalmol Vis Sci* 27:1199–1204.
31. Sobkowicz HM, Slapnick SM, August BK (1995) The kinocilium of auditory hair cells and evidence for its morphogenetic role during the regeneration of stereocilia and cuticular plates. *J Neurocytol* 24:633–653.
32. Engström B, Flock A, Borg E (1983) Ultrastructural studies of stereocilia in noise-exposed rabbits. *Hear Res* 12:251–264.
33. Jones C, et al. (2008) Ciliary proteins link basal body polarization to planar cell polarity regulation. *Nat Genet* 40:69–77.
34. Verghese E, et al. (2009) Renal primary cilia lengthen after acute tubular necrosis. *J Am Soc Nephrol* 20:2147–2153.
35. Hellman NE, et al. (2010) The zebrafish foxj1a transcription factor regulates cilia function in response to injury and epithelial stretch. *Proc Natl Acad Sci USA* 107: 18499–18504.
36. Schneider L, et al. (2010) Directional cell migration and chemotaxis in wound healing response to PDGF-AA are coordinated by the primary cilium in fibroblasts. *Cell Physiol Biochem* 25:279–292.
37. Pitaval A, Tseng Q, Bornens M, Théry M (2010) Cell shape and contractility regulate ciliogenesis in cell cycle-arrested cells. *J Cell Biol* 191:303–312.

Appendix 4

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Review

# Cilium, centrosome and cell cycle regulation in polycystic kidney disease ☆

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ABSTRACT

Polycystic kidney disease is the defining condition of a group of common life-threatening genetic disorders characterized by the bilateral formation and progressive expansion of renal cysts that lead to end stage kidney disease. Although a large body of information has been acquired in the past years about the cellular functions that characterize the cystic cells, the mechanisms triggering the cystogenic conversion are just starting to emerge. Recent findings link defects in ciliary functions, planar cell polarity pathway, and centrosome integrity in early cystic development. Many of the signals dysregulated during cystogenesis may converge on the centrosome for its central function as a structural support for cilia formation and a coordinator of protein trafficking, polarity, and cell division. Here, we will discuss the contribution of proliferation, cilium and planar cell polarity to the cystic signal and will analyze in particular the possible role that the basal bodies/centrosome may play in the cystogenetic mechanisms. This article is part of a Special Issue entitled: Polycystic Kidney Disease.



**Contents**



## 1. Introduction

Hereditary cystic kidney diseases comprise a heterogeneous group of monogenic disorders [1]. In some instances the bilateral development of multiple fluid-filled cysts in kidneys is part of a more complex syndromic clinical manifestation, whereas in others it is a distinctive feature of the disease and an important cause of end stage kidney disease. We will focus on the latter disorders, hereafter referred to as polycystic kidney disease.

Polycystic kidney disease is characterized by the hyperproliferation of tubular epithelial cells, the alterations of their fluid secretion functions, and changes in the extracellular matrix deposition and fibrosis, all of which profoundly alter the organ architecture and impair renal function. Autosomal dominant and autosomal recessive forms of polycystic kidney disease have been recognized with an incidence of 1:800 and 1:20,000, respectively.

Autosomal dominant polycystic kidney disease (ADPKD) is caused by the dysregulation of the PKD1 or PKD2 genes, which code for polycystin-1 (PC1) and polycystin-2 (PC2), respectively. PC1 and PC2 may form a complex through the interaction of the respective carboxyl termini, thus establishing reciprocal regulatory functions. Consequently, regardless of the genotype, the clinical manifestations of ADPKD largely overlap, with few notable exceptions: on average, individuals with mutation in the PKD1 gene reach end stage kidney disease 20 years earlier than patients carrying mutations in the PKD2 gene, and PKD2 mutations result in more severe disease in males than in females.

Autosomal recessive polycystic kidney disease (ARPKD) results from mutations in the polycystic kidney and hepatic disease 1 gene (PKHD1), encoding fibrocystin/polyductin (FPC) [2,3]. ARPKD generally manifests earlier in life with the most severe cases resulting in

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perinatal or neonatal death. In addition, collecting duct ectasia results in cysts that remain connected with the nephrons of origin. Unlike ADPKD, in which cysts are prevalent in the collecting ducts but may develop everywhere along the nephron, in ARPKD cystogenesis is restricted to the collecting ducts.

PC1 is a large integral membrane protein with receptor-like structural characteristics [4], which undergoes a complex Notch-like processing [5,6]. Abundant evidence supports the role of the PC1 carboxyl terminus in signaling mechanisms. The C terminal tail of PC1 contains phosphorylation sites for different tyrosine and serine/threonine kinases [7] and a domain for the interaction with G proteins and the activation of the JNK/AP1 pathway [8,9]. Importantly, in response to changes in mechanical stimulation, the carboxyl terminal tail undergoes regulated intramembrane proteolysis and translocates into the nucleus to activate the AP1 pathway through a process negatively regulated by PC2 [10].

PC2 is a $Ca^{2+}$ regulated, non-selective cation channel that shares sequence and structure similarities with the superfamily of transient receptor potential channels [11–15]. PC2 is expressed predominantly in the ER, but it is also found in the Golgi, the plasma membrane, and on the cilium where with PC1, and likely FPC, it forms a mechanosensor complex that controls $Ca^{2+}$ influx in response to flow [16,17]. On the plasma membrane, PC2 only partially co-localizes with PC1 and adhesion complexes, suggesting that it may function independently as homodimer or participate in different complexes with other members of the TRP family, thus expanding the functional characteristics of these channels [16]. The loose interaction of PC2 with PC1 and adhesion complexes may be important to confer PC2 more dynamic mechanosensorial properties independent of or opposed to PC1. For example, *situs inversus*, the phenotype with reversed orientation of visceral organs, is associated with *Pkd2* but not *Pkd1* knockout mouse models, indicating the independent mechanosensing function of PC2 in the nodal cilia [18]. In the case of stretch-activated ion channels PC1 and PC2 exert opposing effects with PC2 inhibiting channel opening and PC1 reverting this suppression [19]. Though many aspects of the regulation of PC2 function remain unclear, the growing evidence of its multiple interactions with cytoskeleton organizing proteins supports its $Ca^{2+}$-dependent mechanosensorial role at different cellular compartments (for a comprehensive review, see references [15,20,21]). Interestingly, the subcellular localization of PC1 at the cell adherens, desmosomes, focal adhesions, and cilia provides the proximity with cytoskeletal components, suggesting a possible role of PC1 in the control of cytoskeleton rearrangement (Fig. 1) [22–25].

Similar to PC1, FPC undergoes a complex proteolytic process at the ciliary membrane [26,27]. The large ectodomain is cleaved by a proprotein convertase and remains tethered to the carboxyl stalk via

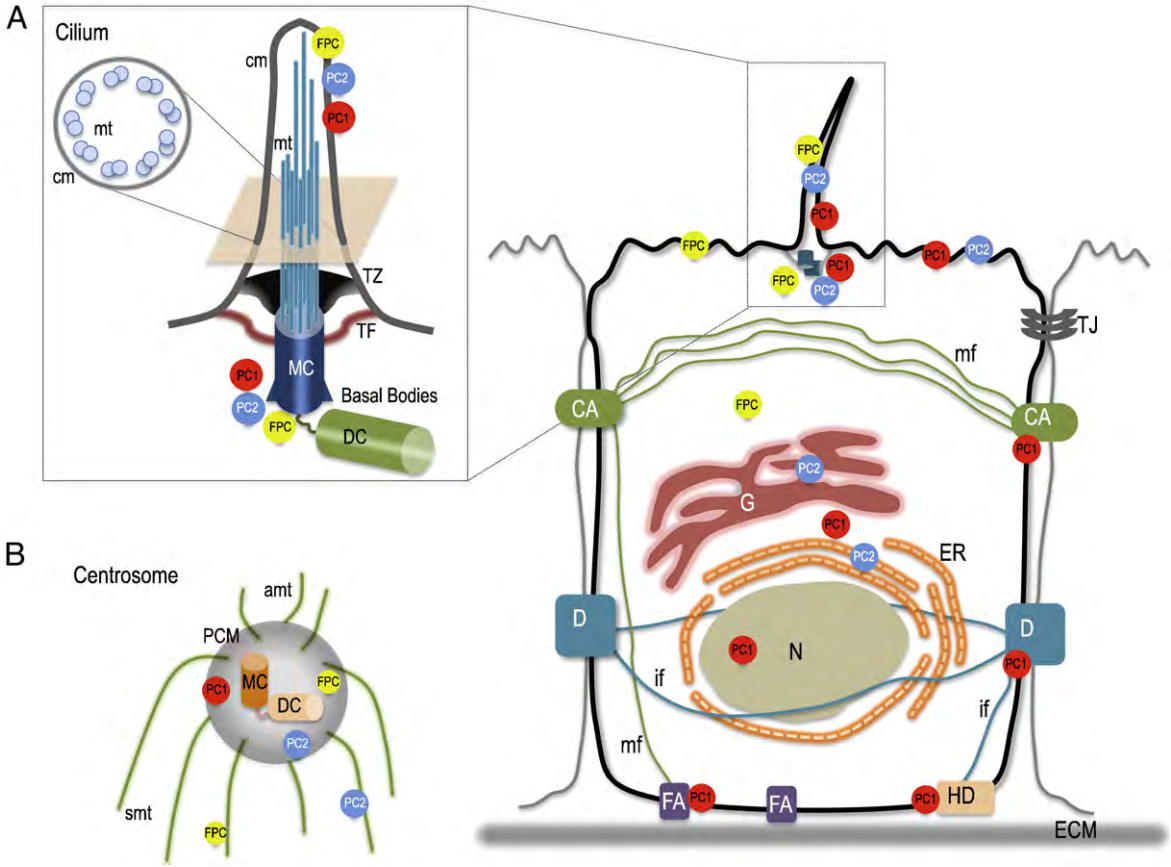


**Fig. 1.** Subcellular localization of PC1, PC2, and FPC. (A) Polycystic proteins localize to multiple compartments within the cell including the cilium in which they form a $Ca^{2+}$ non-selective channel whose activity is essential during renal morphogenesis. In the kidney, the cilium protrudes from the apical side of renal epithelial cells into the lumenal space. The cilium is supported by nine doublets of microtubules that nucleate from the basal body, a specialization of the mother centriole (MC), at the base of the cilium (1A, inset). (B) Following cilium resorption, pericentriolar material (PCM) organizes around the centrioles to form the centrosome. Microtubules emanating from the centrosome maintain cellular structure and are required for multiple cellular functions including spindle organization and cytokinesis. Polycystic proteins also localize to the centrosome and both PC2 and FPC are found to associate with the spindle microtubules during cell division. While the ciliary localization of polycystic proteins is important for fluid flow sensing, their function on the centrosome and mitotic spindle remains obscure. Similarly unclear is whether the localization at cell–cell and cell–matrix contacts plays a role in tension sensing and cytoskeletal rearrangement. N, nucleus; G, Golgi apparatus; ER, endoplasmic reticulum; CA, cell adherens; D, desmosomes; HD, hemidesmosomes; FA, focal adherens; ECM, extracellular matrix; TJ, tight junction; mt, microtubules; mf, actin microfilaments; if, intermediate filaments; MD, mother centriole; DC, daughter centriole; TZ, transition zone; TF, transition fiber; PCM, pericentriolar matrix; cm, ciliary membrane; amt, astral microtubules; smt, spindle microtubules.

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disulfide bonds. Shedding of the ectodomain occurs concomitantly to the regulated intramembrane proteolysis that releases the intracellular cytoplasmic tail that then traffics to the nucleus or nucleolus. This process appears to be dependent on intracellular $Ca^{2+}$ release, but it remains unknown whether a ligand or a mechanical change triggers the shedding of the ectodomain [26]. Similarly, the possible paracrine signaling function of the shed domain and the implication of the nuclear translocation on gene regulation remain unclear, though the Notch-like regulation and the ciliary localization of the process suggest that it may be involved in the maintenance of nephron architecture.

Excellent reviews have presented and discussed the characteristics and functions of polycystic genes and their encoded proteins in detail [28–31]. Here we will review the early events of renal cystogenesis and the relationship of polycystic proteins with the centrosome, its association with the cilium and its function in cell cycle control.

## 2. Proliferation of cystic cells

Cell hyperproliferation underlying continuous expansion of the cysts and renal enlargement is a hallmark of ADPKD and ARPKD and a determinant of renal failure [32,33]. Under normal conditions the mitotic index of the adult kidney is very low. However, in renal tissues from ADPKD or ARPKD patients, as well as from *Pkd1* or *Pkd2* mutant animal models, nuclei positive for proliferating cell nuclear antigen (PCNA) and Ki67 mitotic markers are readily detectable [34–36]. In fact, multiple mitogenic pathways may be constitutively activated in polycystic kidney disease as a consequence of altered $Ca^{2+}$ homeostasis or abnormal protein trafficking.

Defects in PC2 $Ca^{2+}$ channel activity that lead to low intracellular $Ca^{2+}$ concentration, aberrant G-protein signaling by PC1 dysregulation [8,37], and decreased cyclic nucleotide catabolism [38] may contribute to the accumulation of cAMP and the abnormal activation of the $Ca^{2+}$ inhibitable adenyl cyclase 5/6. Cystic cells proliferate in response to increased cAMP levels [39,40] and the activation of the PKA/B-Raf/MAPK pathway [41], in contrast to normal primary renal epithelial cells, whose growth is inhibited by cAMP [42]. Cyst expansion then accelerates partly through a mechanism promoting chloride -driven fluid secretion [43,44].

Altered protein trafficking may also contribute to cystogenic signals as in the case of the mislocalization of EGF receptors in renal epitelia. The EGF receptor (EGFR/HER1) is normally expressed apically during the embryonic mammalian kidney development, but its localization shifts to the basal side in the adult organ. In ADPKD and ARPKD, however, the EGFR/HER1 expression is increased and mislocalized to the apical membrane where it results in a paracrine loop of persistent stimulation by its ligand released in the filtrate or in the cystic fluid [45–47]. Other dedifferentiating processes characteristic of cystic cells may further reinforce this autostimulatory mechanism. For instance, the expression of ErbB2/Neu/HER2, a member of the EGFR superfamily, is developmentally regulated and restricted to the embryonic kidneys. However, the re-expression ErbB2/Neu/HER2 in the adult ADPKD renal epithelia allows it to heterodimerize with EGFR/HER1 on the apical membrane [48]. The interference with the autocrine/paracrine EGF/EGFR stimulatory loop reduced cystic lesions in organ culture [49], slowed down cyst expansion and ameliorated polycystic kidney disease in different, though not all, animal models [50–53].

Other proliferative pathways may also be activated. In particular, the evidence of the activation of the mammalian target of rapamycin (mTOR) signaling in the cyst lining cells of the kidneys from different mouse models of renal cystic disease (*MAL*, overexpressing myelin and lymphocyte protein; and the IFT88 hypomorph, *orpk*) and in human ADPKD specimens suggests that this may be a common pathway underlying cystic proliferation [54] (for an extensive review, see [55]).

The serine/threonine kinase mTOR is the key component of the multiprotein complexes mTORC1, which positively controls protein translation, cell metabolism and proliferation, and mTORC2, which is involved in actin cytoskeleton organization and cell survival [56,57]. The activation of mTORC1 is suppressed by the heterodimer of hamartin and tuberin, encoded by the *TSC1* and *TSC2* genes, respectively. Stimulation of the PI3kinase/Akt or ERK pathways leads to the phosphorylation-mediated inhibition of TSC2/tuberin and the activation of mTORC1 [58,59]. Tuberin and PC1 functionally cooperate to regulate the mTOR pathway. PC1 interacts with tuberin [54], sequesters it on the membrane and protects it from Akt phosphorylation, thus suppressing the activation of mTORC1 [60]. Conversely, tuberin is necessary for the proper localization PC1, as seen in Eker rats that carry a homozygous mutation of the *Tsc2* gene. In the absence of functional tuberin, PC1 accumulates in the Golgi and fails to properly traffic to the lateral cell membrane, demonstrating that tuberin is necessary for correct PC1 localization [61].

In various animal models of polycystic kidney disease, a significant reduction of cystic growth has been obtained by pharmacologically preventing the cAMP increase, $Ca^{2+}$ imbalance, EGF stimulation, mTOR activation [62–67] or by inhibiting cell cycle progression with the cyclin-dependent kinase inhibitor, roscovitine [68]. These *in vivo* results have provided the rationale for different experimental therapeutic approaches that are currently under investigation [69]. However, recently concluded clinical trials that tested the efficacy of mTOR inhibitors (rapamycin/sirolimus and the analog everolimus) on ADPKD patients at different stages of the disease yielded disappointing results. Treatment with these inhibitors showed no improvement in the renal function, despite a transient reduction in total kidney volume in patients with a more advanced stage of disease [70,71]. Unlike the findings from a shorter study with sirolimus on fewer patients [72], these trials also indicated that both mTOR inhibitors presented considerable side effects that severely limit their therapeutic value for ADPKD, even when administered at doses far lower than those used in the animal models.

A further detailed analysis of these studies may help explain some of the differences within the human studies and the discrepancies with the experimental data on animal models as commented in references [73,74]. In light of the remarkably promising preclinical results, it would be premature to interpret the discouraging results of the human trials to confute the validity of targeting the mTOR pathway in cystic diseases. Rather, it may be necessary to explore alternative strategies in which mTOR inhibition is part of a combination therapy or in which mTOR inhibitors could be specifically targeted to the kidney.

A smaller clinical trial on ADPKD patients was also conducted to assess the efficacy of octreotide, a long-lasting somatostatin analog, that inhibits the intracellular accumulation of cAMP in renal epithelia [75]. In this 12-month study, results similar to those with mTOR inhibitors were obtained: octreotide arrested the increase of kidney volume but failed at improving kidney function. Differently from mTOR inhibitors, however, octreotide appeared to be well tolerated with no serious adverse effects.

Overall, these clinical trials underscore the complexity and variability of the disease progression, and question the use of kidney volume change as a surrogate marker of organ function [32,33,73]. They also suggest that at advanced stages of the disease, cell proliferation is dissociated from cellular and organ function. As such, proliferation may have different roles at different stages of cystogenesis. Findings of proliferating cells in normal tubular epithelia surrounding cysts suggest that cell growth is an early event in the cystogenic transformation. Nevertheless, no cysts derive from the active proliferation during normal organ morphogenesis, and active growth of renal carcinoma cells does not necessarily result in cyst formation. Therefore, it remains difficult to establish whether the activation of these pathways represents the cystogenic trigger or if it supports cyst expansion.

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## 3. Cilium and cystogenesis

The observation that cystic proteins localize on the primary cilium and basal body [76–78] provided new insights into the mechanisms of renal cystic diseases. The intense focus on the cilium that followed unveiled the genetic determinants of numerous complex diseases that define a new class of disorders collectively referred to as ciliopathies (for comprehensive reviews, see references [79–82]).

The primary cilium is a highly compartmentalized organelle present in most cell types that functions as a sensor of extracellular environmental cues. It is formed as a single protrusion of the plasma membrane supported by the axoneme, a cytoskeletal component that is assembled as a ring of 9 microtubule doublets arranged tangentially to the center in a configuration known as 9 + 0 (Fig. 1A, inset). Defects in cilia formation result in complex phenotypes, which invariably include cystic kidneys [83]. In renal epithelia, cilia convert mechanical force of fluid flow into cellular functions [84]. PC1, PC2 and FPC are expressed in renal primary cilia where they are a part of a mechanosensor complex that translates the ciliary bending induced by flow into $Ca^{2+}$ influx [85–87]. Their functional role was supported by observation that STAT6, whose ciliary localization depends on flow stimulation, is part of a complex that includes the cleaved carboxyl terminus of PC1 and the transcriptional coactivator P100. As the carboxyl tail of PC1 is proteolytically cleaved, the complex translocates into the nucleus and activates gene expression, thereby linking mechanical stimulation of the cilium by urine flow and cellular responses [88]. However, impaired mechanosensation of cilium as a primary defect in cystogenesis was challenged by the work on conditional knockout models of the intraflagellar transport *Ift88* (polaris) and the *Kif3a* subunit of kinesin-2 genes, which are essential for ciliogenesis [89]. While the deletion of *Ift88* or *Kif3a* during gestation prevented cilia formation and resulted in severe cystic disease within 2 weeks after birth, deletion of either gene in the adult animals did not immediately result in detectable cystic phenotype, despite the cilia ablation. Eventually, mild renal cyst formation was observed 6 months after the knockout, revealing different requirements of ciliary function during renal development and in the maintenance of adult kidney [89]. These results indicated that cilia are dispensable in adult mice and that other components may participate in the cystic process.

Interestingly, the conditional models of *Pkd1* inactivation similarly displayed greater susceptibility of young mice to develop severe cystic kidney disease as compared to the adult mice. These mouse models offered the opportunity to investigate the early cystogenetic events following the depletion of PC1 [90–92]. A detailed analysis of perinatal *Pkd1* inactivation demonstrated that the deletion of the gene within day P13 led to extensive cystogenesis and kidney enlargement, whereas inactivation of *Pkd*1 from day P14 onward resulted in late onset cystic kidney disease [91]. These observations uncovered a window of susceptibility, which corresponds with the completion of mouse nephrogenesis when proliferation is actively ongoing and a specifically timed brake point. However, the study also showed that although proliferation abruptly decreased after day P14, it remained significantly higher at P16 as compared to the adult kidney. Nevertheless the course of cystic disease was comparable in the P16 and older mice, suggesting that proliferation *per se* may not be sufficient to trigger the cystogenic change [91]. As the brake point was characterized molecularly by a change in gene expression pattern consistent with a developmental switch, it was proposed that components of an early developmental program could in fact be the cystogenic triggers. Such a program may be recalled during the re-epithelization process that follows renal injury. In support of this notion, in adult kidneys in which *Pkd1* is conditionally inactivated or in which no cilia can be formed because of the conditional *Kif3a* excision, the cystic phenotype can be accelerated by the induction of ischemia/reperfusion injury or pharmacological nephrotoxicity [36,93,94]. In addition, although cell growth occurs rapidly following injury, it reverts to control levels before cystic expansion, again suggesting that proliferation cannot be the only cystogenic switch [94]. Nevertheless, even though proliferation may not be sufficient as the sole cystogenic trigger, it may yet provide the necessary context for such a trigger to arise as indicated in recent experiments on the conditional inactivation of the *Hnf1b* gene, which encodes a transcription factor involved in the expression of genes that include *Pkd2*, *Pkhd1* and *UMOD* (encoding uromodulin). Similar to the *IFT88*, *Kif3a*, and *Pkd1* models, the pre- or perinatal conditional inactivation of *Hnf1b* leads to rapid polycystic kidney disease, while the ablation of *Hfn1b* in the adult leads to slow onset cystic disease that can turn into rapidly progressing disease following renal injury. Careful analysis of proliferating cells using BrdU showed that tubular dilatation coincided with the regenerative proliferation burst and the loss of mitotic orientation only in the mutant dividing cells and not in those of wild type kidneys [95]. Therefore, cell proliferation may create the conditions for the cystogenic switch, which may include defects in oriented cell division, planar cell polarity (PCP), and changes in the centrosome positioning [93–95].

## 4. Role of planar cell polarity in cystogenesis

The organization and asymmetric distribution of protein content that cells maintain in parallel to the epithelial plane is called planar cell polarity (PCP). The mechanisms of PCP are fundamental for the developmental patterning of both invertebrates and vertebrates [96] and are regulated by the non-canonical Wnt pathway (for a comprehensive review of the Wnt signaling in cystic diseases, see reference [97]).

During kidney development, the spindle of the dividing cell organizes with an orientation parallel to the axis of the elongating tubule, revealing an intrinsic cell polarity. The evidence of a link between cilia and PCP came from the observation that the ciliary protein inversin, the product of the NPHP2 gene whose mutations cause nephronophtisis, functioned as a switch from the canonical to the non-canonical Wnt pathway [98]. Whether PCP in turn played a role in cystic disease was first observed in kidneys in two rodent renal cystic models: the mouse with inactivation of the Tcf2/HNF1β transcription factor [99], and the *pck* rat, which carries a mutated *PKHD1* gene ortholog [100]. In both cystic models a significant number of spindles in the dividing cells of the kidneys were misaligned, suggesting that the loss of proper spindle orientation and planar cell polarity are linked to cystogenesis [101].

Recently, a direct proof of the role of PCP in renal cystic development was provided by the knockout mouse model of *Fat4* gene, which encodes a PCP protein of the proto-cadherins family [102]. Homozygous $Fat4^{-/-}$ mutants died at birth but displayed multiple characteristics of PCP protein defects including anomalies in the elongation of the cochlea and disruption of hair cell organization in the organ of Corti. $Fat4^{-/-}$ mutants also displayed smaller kidneys with dilated and shorter tubules and significant defects in oriented cell division. Crossing $Fat4^{-/-}$ mice with mutants for other PCP components, *Vangl2* and *Fjx1*, exacerbated the cystic phenotype [102]. Together with the ciliary localization of FAT4, these findings further strengthened the link between PCP and cilium during cystogenesis. The interdependence of PCP and ciliary function is also supported by observations with other PCP core proteins, Dishevelled and Vangl2. Dishevelled is involved in the docking of the centrioles/basal bodies to the apical membrane that precedes ciliogenesis [103], and Vangl2 is required for the asymmetric positioning of motile cilia in cells of zebrafish neural tube [104]. Furthermore, the fluid flow influences centrioles' movement and contributes to the orientation of motile cilia in conjunction with PCP in ependymal cells [105,106].

It should be noted, however, that more recent reports question the role of oriented cell division as a primary cause of cystogenesis. In the hypomorphic mutant for *Wnt9b*, whose expression is required for

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renal morphogenesis, cystogenesis starts *in utero*, leading to the development of grossly cystic kidneys within a month of age [107]. The analysis of the embryonic renal development revealed that during the period from E13.5 to P1, tubules lengthen through the movement of the cells that assume an elongated shape parallel to the tubule axis in a process of convergent extension, which is dependent on PCP and the activation of the Rho/Jnk signaling pathway. The impairment of this process in the *Wnt9b* mutants alters tubule diameter and triggers cyst formation. Interestingly, until P1, cell division appeared similarly misoriented in both *Wnt9b* mutants and wild type mice, suggesting that defects in oriented cell division alone cannot account for *in utero* cystogenesis [107]. Moreover, a study in *Pkd1*, *Pkd2* and *Phkd1* mouse mutants showed that changes in oriented cell division did not precede cystogenesis, but rather followed the cystic transformation [108]. While challenging the defects of oriented cell division as a driver of cystogenesis, these results nevertheless emphasize the role of PCP in cystogenesis.

## 5. Centrosome and cell cycle

The basal bodies located at the base of the cilium are a morphological specialization of the centrioles/centrosome, specifically the mother centriole from which the axoneme emanates to support the formation of the primary cilium (Fig. 1). Functionally, the basal bodies participate in the intraflagellar transport (IFT) through the organization of the transition zone and the control of vesicles trafficking to and from the cilium [109], thereby coupling the cilium and centrosome functions. The essential role played by the centrosome in coordinating the ciliary and PCP crosstalk is further emphasized by the alteration of the Wnt signaling following the disruption of basal bodies in zebrafish *bbs4* morphants [110].

The centrioles/centrosome serve as the microtubule-organizing center (MTOC), and thus play a major role in the spatial organization of the microtubular network required for not only the formation of primary cilia, but also cell polarity, migration, trafficking of cytoplasmic organelles, and organization of the mitotic spindle [111]. Because of these essential functions that it underlies, the centrosome integrity and duplication are tightly controlled. In most cells, under normal conditions the centrosome divides only once per cell cycle through a mechanism coupled to the cell cycle progression, so that each daughter cell receives only one centrosome [112,113]. Reciprocal interactions exist between IFT and centrosomal proteins to regulate their trafficking and localization. For example, IFT20 shuttles between the Golgi and the cilium, and is required for the localization of pericentrin to the centrosome [114,115]. Conversely, reduced expression of pericentrin also lowers the levels of IFT20, IFT88, IFT57 and PC2 in centrioles and inhibits cilia formation [115].

In cells preparing to cycle, the cilium is reabsorbed, leaving the basal bodies/centrioles free to anchor to the cell cortex and to be ready for centrosome duplication and the subsequent organization of the microtubule rearrangement that is required for the assembly of the spindle, mitosis, and cytokinesis. Cilium resorption may allow redistribution of ciliary components to the centrosome that can affect the cell cycle progression. For instance, IFT88/polaris remains tightly associated with the centrosome and modulates the G1–S transition by titering out Che-1, an inhibitor of the growth suppressor function of Rb [116]. Consequently, interfering with various centrosome proteins leads to the p53-dependent block of cell cycle progression from G1 to S and failure to assemble cilia [117,118]. p53 is also a centrosomal protein, and its depletion increases centrosome amplification [119]. The control of cell cycle progression and restriction of centrosome overduplication by p53 is exerted partly via the transactivation of p21 and the direct association of p53 with the centrosome [120–122].

Both PC1 and PC2 exert a direct effect on cell cycle and centrosome duplication. The heterologous expression of PC1 or PC2 arrests the cell cycle in G1 through different mechanisms that converge on the induction of the cyclin-dependent kinase (Cdk) inhibitor p21 and the inhibition of Cdk2 activity [123,124]. In the case of PC1, the expression of p21 results from the activation of the JAK2-dependent phosphorylation of STAT1, but not of p53 [123]. In contrast, PC2 functions by binding to Id2, a member of the helix–loop–helix (HLH) family of transcriptional regulators that antagonize basic HLH transcription factors that are involved in the control of cell cycle progression. The interaction with PC2 sequesters Id2 in the cytosol, thus preventing its translocation into the nucleus where it suppresses p21 transcription [124]. PC1 and PC2 exert a reciprocal control on the activation of these pathways. The physical interaction of PC1 with JAK2 is dependent on the presence of PC2 as a cofactor, whereas PC1 phosphorylation of PC2 is required for its interaction with Id2. Conversely, depletion of PC1 or PC2 results in faster G1 to S progression [124–126] and reduced expression of p53 in HEK293 cells [125]. Lowered p53 expression is also observed in embryonic kidneys of *Pkd1* −/− mice [127], albeit in this case it is difficult to determine whether such downregulation is an effector or a consequence of the cystogenic transformation.

Polycystic proteins localize on the centrosome and are important to maintain centrosome integrity (Fig. 2). The inhibition of PC1 expression induces centrosome amplification *in vitro*, and supernumerary centrosomes were observed both in the kidneys of *Pkd1* conditional knockout animal model and in human renal tissue from ADPKD patients *in vivo* [128]. These centrosomes appeared fully functional, as they were able to organize multipolar spindles. However, the cells dividing with aberrant mitotic spindles entered mitotic catastrophe or produced genetically unstable progeny, characterized by significant apoptosis and aneuploidy [129,130]. Amplified centrosomes were noted on seemingly normal tubular cells, suggesting that centrosome aberrations may be an early event in the cystic conversion [128]. Similarly, centrosome amplification was also reported in fibroblast cell lines derived from *Pkd2* transgenic mice and in mesenchymal cells of *Pkd2* knockout embryos [131], indicating that PC2 dysregulation also affects centrosome integrity. Polycystins' broad tissue distribution and the effects of interference of PC1 or PC2 in centrosome integrity in non-renal cells suggest that polycystins play a fundamental role in the mechanisms controlling centrosome duplication and that centrosomal aberrations may be important in cystic development.

More recently, at least some FPC isoforms have also been shown to be required for the maintenance of centrosome integrity and proper spindle assembly [132]. Similarly to PC2, FPC is found on the spindle during cell division, but the mechanisms controlling its localization remain unknown. The spindle localization of PC2, however, was shown to require the interaction with Diaphanous (mDia)-related formin 1, mDia1 [133], a protein involved in actin polymerization and microtubule stabilization [134]. Depletion of mDia1 coincides with the loss of PC2 localization from the spindle and a decreased $Ca^{2+}$ release in mitotic cells. The function of PC2 on the spindle is unclear, but the interaction of PC2 with the actin bundling protein α-actinin and with the microtubule-dependent motor kinesin-2 subunit KIF3A, both of which activate PC2 channel activity *in vitro*, lends support to the intriguing possibility that PC2-mediated $Ca^{2+}$ transport may function in the cytoskeletal remodeling required for cell division [21,135,136]. Although the spindle localization of PC1 is unclear, its presence on the centrosome along with FPC may be important in the reconstitution and regulation of the PC2 $Ca^{2+}$ channel activity [87,132,137]. Overall, these observations underline the interdependence of cilium, centrosome, and cytoskeletal rearrangement.

The mechanisms contributing to centrosome amplification remain speculative, but it might involve the altered expression of p53 and/or cyclin-A, as observed in PC1-deficient cells [125,138], as well as imbalanced $Ca^{2+}$ homeostasis. Centrosome amplification can occur following cytokinesis failure or by reiterated centriole duplication within the same cycle. Evidence of multinucleation and enlarged nuclei in PC1- or PC2-deficient cells suggests that supernumerary

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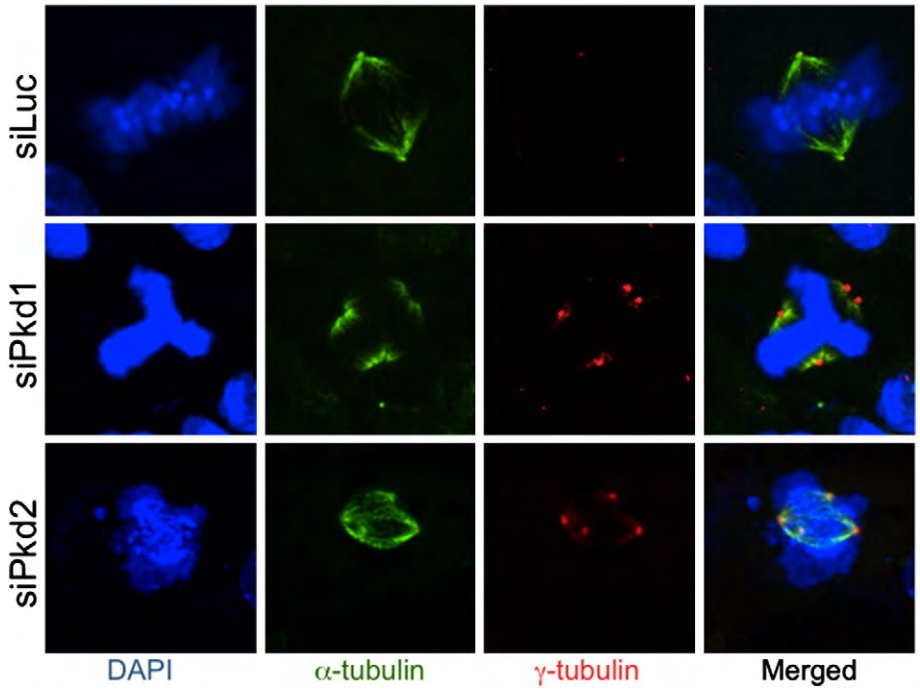


**Fig. 2.** Centrosome amplification following the suppression PC1 or PC2 expression. Centrosome amplification and mitotic spindle abnormalities occur rapidly after the knockdown of *Pkd1* or *Pkd2*. Shown are MDCK cells and IMCD3 cells 3 days following the transduction with a lentivirus constitutively expressing the shRNAs specific for Pkd1 and Pkd2, respectively. IMCD3 cells (or MDKC cells, not shown) transduced with control lentivector expressing shLuc against luciferase (siLuc) maintain normal mitosis. Cells were immunostained with anti-α-tubulin and anti-γ-tubulin to specifically detect microtubules (green) and centrosomes (red), respectively, and then counterstained with DAPI to visualize DNA (blue).

centrosomes may result from endoreduplication. Cytokinesis depends on the accumulation of $Ca^{2+}$ stores to the furrow and on the proper $Ca^{2+}$ release before abscission [139]. It remains to be determined whether the reciprocal interaction of PC2 (or a polycystins complex) with cytoskeletal components has any function on this $Ca^{2+}$ regulation. Furthermore, both centrosome duplication and cell growth processes depend on increased $Ca^{2+}$ transients from internal $Ca^{2+}$ stores [140], a requirement that seems to be at odds with the decreased intracellular $Ca^{2+}$ content in polycystic cells [141,142]. However, experiments on HEK293 cells indicate that PC1 negatively regulates non-capacitative $Ca^{2+}$ entry (NCCE) channels and that cell proliferation upon PC1 knockdown is sustained by an NCCE-dependent increase in $Ca^{2+}$ oscillations [143]. Hence, it is possible that changes in the frequency and amplitude of $Ca^{2+}$ oscillations may support also centrosomal amplification or centriole reduplication [144].

The centrosomal defects extend to other diseases with renal cystic manifestations. Loss of hamartin, the product of the *TSC1* gene whose mutations cause tuberosclerosis, also leads to centrosome amplification [145]. The depletion of the centrosomal Mks1 or Mks3/meckelin proteins, which are mutated in the autosomal recessive Meckel-Gruber syndrome, results in centrosome amplification and, in the case of Mks3, in multiciliation [146]. Renal cysts develop following the loss of IFT20, which results in cilia ablation, centrosome amplification with loss of centrosome positioning, and mitotic spindle misorientation [147]. It will be of interest to determine whether centrosome defects are common to other renal cystic diseases.

Centrosome aberrations occur early after the inhibition of polycystic proteins and, similar to ciliary defects, they may be a common denominator in renal cystic disease. A causative role of centrosome defects in cystogenesis is difficult to establish, but its expected consequences are consistent with all the findings characteristic of ADPKD cells. Errors in centrosome duplication may result in the formation of monopolar or multipolar spindles, aberrations associated with chromosome missegregation, genomic instability, and apoptosis. Cells that accumulate excessive genomic damage/imbalance become apoptotic, whereas others may survive carrying abnormal karyotypes [148,149], and altered physiological functions. A kidney-specific interference with effectors of the centrosome duplication process will be required to establish a causal link between centrosome anomalies and renal cystic development.

## 6. Conclusions

As intense research has focused on cystic cells, we have a better understanding of the mechanisms that support cystic expansion, including alterations of calcium homeostasis and changes in protein trafficking and interactions, which sustain the constitutive activation of mitogenic pathways. On the other hand, the signals (or lack thereof) that trigger the cystic conversion are unknown and the mechanisms underlying the early cystogenic events are just emerging in a picture of increasing complexity.

The view of the cilium as sensor of fluid flow has expanded to the regulation of planar cell polarity and defects in PCP-controlled mechanisms, convergent extension, and oriented cell division have been indicated as possible cystogenic triggers. However, it seems that ciliary functions and PCP in the cystogenic conversion cannot be clearly separated, as they exert a reciprocal regulation. Docking of centrioles/centrosome to the cortex is essential for the formation of the basal bodies and ciliogenesis as well as for the establishment of the spindle pole position [150]. Therefore, a cystogenic trigger driven by centrosome amplification is also conceivable as the presence of supernumerary centrioles, caused by the depletion or malfunction of different cystic proteins, can produce conflicting cues leading to improper attachment, misalignment of the spindle axis, or altered cilium positioning. These effects may be exposed by the dysregulation of cell cycle progression in cells with amplified centrosomes.

Very important has been the finding that a developmental switch limits the cystogenic susceptibility to ciliary defects to a period of time

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largely overlapping with the completion of murine renal morphogenesis [91]. Whether PCP mutants are similarly constrained remains to be demonstrated, and experiments with conditional inactivation of PCP genes may provide a clue on whether and how ciliary and PCP functions follow an order of succession in cystogenesis. In the adult, tubular epithelia injury reestablishes the susceptibility to cystogenesis, although it has not been determined whether this depends on the reactivation of the same renal morphogenetic developmental program. Both early development and repair processes are characterized by the need for cell proliferation. Since proliferation shows a biphasic curve, that is, it subsides before starting again in the cystic cells, it cannot be the main cystogenic trigger [94]. Nevertheless, proliferation appears necessary for the trigger to be set off [95].

While we are gaining a better understanding of multiple cellular processes and cell components that play a role in cystogenesis, a unifying pathogenetic mechanism is still missing, largely due to our incomplete knowledge of the workings of polycystic proteins. Further efforts will be necessary to integrate the functions of cilium/centrosome, PCP, and cell proliferation and to determine the sequence of early events that initiate the cystogenic signal.

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## References

[1] M. Bisceglia, C.A. Galliani, C. Senger, C. Stallone, A. Sessa, Renal cystic diseases: a review, Adv. Anat. Pathol. 13 (2006) 26–56.
[2] L.F. Onuchic, L. Furu, Y. Nagasawa, X. Hou, T. Eggermann, Z. Ren, C. Bergmann, J. Senderek, E. Esquivel, R. Zeltner, S. Rudnik-Schöneborn, M. Mrug, W. Sweeney, E.D. Avner, K. Zerres, L.M. Guay-Woodford, S. Somlo, G.G. Germino, PKHD1, the polycystic kidney and hepatic disease 1 gene, encodes a novel large protein containing multiple immunoglobulin-like plexin-transcription-factor domains and parallel beta-helix 1 repeats, Am. J. Hum. Genet. 70 (2002) 1305–1317.
[3] C.J. Ward, M.C. Hogan, S. Rossetti, D. Walker, T. Sneddon, X. Wang, V. Kubly, J.M. Cunningham, R. Bacallao, M. Ishibashi, D.S. Milliner, V.E. Torres, P.C. Harris, The gene mutated in autosomal recessive polycystic kidney disease encodes a large, receptor-like protein, Nat. Genet. 30 (2002) 259–269.
[4] A.N. Malhas, R.A. Abuknesha, R.G. Price, Interaction of the leucine-rich repeats of polycystin-1 with extracellular matrix proteins: possible role in cell proliferation, J. Am. Soc. Nephrol. 13 (2002) 19–26.
[5] F. Qian, A. Boletta, A.K. Bhunia, H. Xu, L. Liu, A.K. Ahrabi, T.J. Watnick, F. Zhou, G.G. Germino, Cleavage of polycystin-1 requires the receptor for egg jelly domain and is disrupted by human autosomal-dominant polycystic kidney disease 1-associated mutations, Proc. Natl Acad. Sci. USA 99 (2002) 16981–16986.
[6] W. Wei, K. Hackmann, H. Xu, G. Germino, F. Qian, Characterization of cis-autoproteolysis of polycystin-1, the product of human polycystic kidney disease 1 gene, J. Biol. Chem. 282 (2007) 21729–21737.
[7] P.D. Wilson, Polycystin: new aspects of structure, function, and regulation, J. Am. Soc. Nephrol. 12 (2001) 834–845.
[8] S. Parnell, B. Magenheimer, R. Maser, C. Rankin, A. Smine, T. Okamoto, J. Calvet, The polycystic kidney disease-1 protein, polycystin-1, binds and activates heterotrimeric G-proteins in vitro, Biochem. Biophys. Res. Commun. 251 (1998) 625–631.
[9] S.C. Parnell, B.S. Magenheimer, R.L. Maser, C.A. Zien, A.-M. Frischauf, J.P. Calvet, Polycystin-1 activation of c-Jun N-terminal kinase and AP-1 is mediated by heterotrimeric G proteins, J. Biol. Chem. 277 (2002) 19566–19572.
[10] V. Chauvet, X. Tian, H. Husson, D.H. Grimm, T. Wang, T. Hiesberger, T. Hieseberger, P. Igarashi, A.M. Bennett, O. Ibraghimov-Beskrovnaya, S. Somlo, M.J. Caplan, Mechanical stimuli induce cleavage and nuclear translocation of the polycystin-1 C terminus, J. Clin. Invest. 114 (2004) 1433–1443.
[11] Y. Cai, Y. Maeda, A. Cedzich, V.E. Torres, G. Wu, T. Hayashi, T. Mochizuki, J.H. Park, R. Witzgall, S. Somlo, Identification and characterization of polycystin-2, the PKD2 gene product, J. Biol. Chem. 274 (1999) 28557–28565.
[12] P. Koulen, Y. Cai, L. Geng, Y. Maeda, S. Nishimura, R. Witzgall, B.E. Ehrlich, S. Somlo, Polycystin-2 is an intracellular calcium release channel, Nat. Cell Biol. 4 (2002) 191–197.
[13] F.J. Alenghat, S.M. Nauli, R. Kolb, J. Zhou, D.E. Ingber, Global cytoskeletal control of mechanotransduction in kidney epithelial cells, Exp. Cell Res. 301 (2004) 23–30.
[14] M. Köttgen, TRPP2 and autosomal dominant polycystic kidney disease, Biochim. Biophys. Acta 1772 (2007) 836–850.
[15] L. Tsiokas, S. Kim, E.-C. Ong, Cell biology of polycystin-2, Cell. Signal. 19 (2007) 444–453.
[16] M.S. Scheffers, H. Le, P. van der Bent, W. Leonhard, F. Prins, L. Spruit, M.H. Breuning, E. de Heer, D.J.M. Peters, Distinct subcellular expression of endogenous polycystin-2 in the plasma membrane and Golgi apparatus of MDCK cells, Hum. Mol. Genet. 11 (2002) 59–67.
[17] Y. Luo, P.M. Vassilev, X. Li, Y. Kawanabe, J. Zhou, Native polycystin 2 functions as a plasma membrane $Ca^{2+}$-permeable cation channel in renal epithelia, Mol. Cell. Biol. 23 (2003) 2600–2607.
[18] C. Karcher, A. Fischer, A. Schweickert, E. Bitzer, S. Horie, R. Witzgall, M. Blum, Lack of a laterality phenotype in Pkd1 knock-out embryos correlates with absence of polycystin-1 in nodal cilia, Differentiation 73 (2005) 425–432.
[19] R. Sharif-Naeini, J.H.A. Folgering, D. Bichet, F. Duprat, I. Lauritzen, M. Arhatte, M. Jodar, A. Dedman, F.C. Chatelain, U. Schulte, K. Retailleau, L. Loufrani, A. Patel, F. Sachs, P. Delmas, D.J.M. Peters, E. Honoré, Polycystin-1 and -2 dosage regulates pressure sensing, Cell 139 (2009) 587–596.
[20] L. Tsiokas, Function and regulation of TRPP2 at the plasma membrane, Am. J. Physiol. Ren. Physiol. 297 (2009) F1–F9.
[21] X.-Z. Chen, Q. Li, Y. Wu, G. Liang, C.J. Lara, H.F. Cantiello, Submembraneous microtubule cytoskeleton: interaction of TRPP2 with the cell cytoskeleton, FEBS J. 275 (2008) 4675–4683.
[22] Y. Huan, J. van Adelsberg, Polycystin-1, the PKD1 gene product, is in a complex containing E-cadherin and the catenins, J. Clin. Invest. 104 (1999) 1459–1468.
[23] L. Geng, C. Burrow, H. Li, P. Wilson, Modification of the composition of polycystin-1 multiprotein complexes by calcium and tyrosine phosphorylation, Biochim. Biophys. Acta 1535 (2000) 21–35.
[24] M. Boca, L. D'Amato, G. Distefano, R. Polishchuk, G. Germino, A. Boletta, Polycystin-1 induces cell migration by regulating phosphatidylinositol 3-kinase-dependent cytoskeletal rearrangements and GSK3beta-dependent cell–cell mechanical adhesion, Mol. Biol. Cell 18 (2007) 4050–4061.
[25] H. Gao, L.K. Sellin, M. Pütz, C. Nickel, M. Imgrund, P. Gerke, R. Nitschke, G. Walz, A.G. Kramer-Zucker, A short carboxy-terminal domain of polycystin-1 reorganizes the microtubular network and the endoplasmic reticulum, Exp. Cell Res. 315 (2009) 1157–1170.
[26] T. Hiesberger, E. Gourley, A. Erickson, P. Koulen, C.J. Ward, T.V. Masyuk, N.F. Larusso, P.C. Harris, P. Igarashi, Proteolytic cleavage and nuclear translocation of fibrocystin is regulated by intracellular $Ca^{2+}$ and activation of protein kinase C, J. Biol. Chem. 281 (2006) 34357–34364.
[27] J.-Y. Kaimori, Y. Nagasawa, L.F. Menezes, M.A. Garcia-Gonzalez, J. Deng, E. Imai, L.F. Onuchic, L.M. Guay-Woodford, G.G. Germino, Polyductin undergoes notch-like processing and regulated release from primary cilia, Hum. Mol. Genet. 16 (2007) 942–956.
[28] V.E. Torres, P.C. Harris, Autosomal dominant polycystic kidney disease: the last 3 years, Kidney Int. 76 (2009) 149–168.
[29] P.C. Harris, 2008 Homer W. Smith Award insights into pathogenesis polycystic kidney disease gene discovery, J Am Soc Nephrol 20 (2009) 1188–1198.
[30] J. Zhou, Polycystins and primary cilia: primers for cell cycle progression, Annu. Rev. Physiol. 71 (2009) 83–113.
[31] H.C. Chapin, M.J. Caplan, The cell biology of polycystic kidney disease, J. Cell Biol. 191 (2010) 701–710.
[32] J. Grantham, V. Torres, A. Chapman, L. Guay-Woodford, K. Bae, B. King, L. Wetzel, D. Baumgarten, P. Kenney, P. Harris, S. Klahr, W. Bennett, G. Hirschman, C. Meyers, X. Zhang, F. Zhu, J. Miller, Volume progression in polycystic kidney disease, N. Engl. J. Med. 354 (2006) 2122–2130.
[33] J.J. Grantham, A.B. Chapman, V.E. Torres, Volume progression in autosomal dominant polycystic kidney disease: the major factor determining clinical outcomes, Clin. journal Am. Soc. Nephrol. CJASN 1 (2006) 148–157.
[34] T. Nadasdy, Z. Laszik, G. Lajoie, K.E. Blick, D.E. Wheeler, F.G. Silva, Proliferative activity of cyst epithelium in human renal cystic diseases, J. Am. Soc. Nephrol. 5 (1995) 1462–1468.
[35] M.Y. Chang, E. Parker, S. Ibrahim, J.R. Shortland, M.E. Nahas, J.L. Haylor, A.C.M. Ong, Haploinsufficiency of Pkd2 is associated with increased tubular cell proliferation and interstitial fibrosis in two murine Pkd2 models, Nephrol. Dial. Transplant. 21 (2006) 2078–2084.
[36] A. Takakura, L. Contrino, X. Zhou, J.V. Bonventre, Y. Sun, B.D. Humphreys, J. Zhou, Renal injury is a third hit promoting rapid development of adult polycystic kidney disease, Hum. Mol. Genet. 18 (2009) 2523–2531.
[37] P. Delmas, H. Nomura, X. Li, M. Lakkis, Y. Luo, Y. Segal, J.M. Fernández-Fernández, P. Harris, A.-M. Frischauf, D.A. Brown, J. Zhou, Constitutive activation of G-proteins by polycystin-1 is antagonized by polycystin-2, J. Biol. Chem. 277 (2002) 11276–11283.
[38] X. Wang, C.J. Ward, P.C. Harris, V.E. Torres, Cyclic nucleotide signaling in polycystic kidney disease, Kidney Int. 77 (2010) 129–140.
[39] R. Mangoo-Karim, M. Uchic, C. Lechene, J.J. Grantham, Renal epithelial cyst formation and enlargement in vitro: dependence on cAMP, Proc. Natl Acad. Sci. USA 86 (1989) 6007–6011.
[40] K. Hanaoka, W.B. Guggino, cAMP regulates cell proliferation and cyst formation in autosomal polycystic kidney disease cells, J. Am. Soc. Nephrol. 11 (2000) 1179–1187.
[41] T. Yamaguchi, S. Nagao, D.P. Wallace, F.A. Belibi, B.D. Cowley, J.C. Pelling, J.J. Grantham, Cyclic AMP activates B-Raf and ERK in cyst epithelial cells from autosomal-dominant polycystic kidneys, Kidney Int. 63 (2003) 1983–1994.
[42] M. Sutters, T. Yamaguchi, R.L. Maser, B.S. Magenheimer, P.L. St John, D.R. Abrahamson, J.J. Grantham, J.P. Calvet, Polycystin-1 transforms the cAMP growth-responsive phenotype of M-1 cells, Kidney Int. 60 (2001) 484–494.
[43] F.A. Belibi, G. Reif, D.P. Wallace, T. Yamaguchi, L. Olsen, H. Li, G.M. Helmkamp, J.J. Grantham, Cyclic AMP promotes growth and secretion in human polycystic kidney epithelial cells, Kidney Int. 66 (2004) 964–973.

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[44] R. Montesano, H. Ghzili, F. Carrozzino, B.C. Rossier, E. Féraille, cAMP-dependent chloride secretion mediates tubule enlargement and cyst formation by cultured mammalian collecting duct cells, Am. J. Physiol. Ren. Physiol. 296 (2009) F446–F457.
[45] Y. Cai, G. Anyatonwu, D. Okuhara, K.-B. Lee, Z. Yu, T. Onoe, C.-L. Mei, Q. Qian, L. Geng, R. Wiztgall, B.E. Ehrlich, S. Somlo, Calcium dependence of polycystin-2 channel activity is modulated by phosphorylation at Ser812, J. Biol. Chem. 279 (2004) 19987–19995.
[46] J. Du, P.D. Wilson, Abnormal polarization of EGF receptors and autocrine stimulation of cyst epithelial growth in human ADPKD, Am. J. Physiol. 269 (1995) C487–C495.
[47] S.A. Orellana, W.E. Sweeney, C.D. Neff, E.D. Avner, Epidermal growth factor receptor expression is abnormal in murine polycystic kidney, Kidney Int. 47 (1995) 490–499.
[48] S. Wilson, K. Amsler, D. Hyink, X. Li, W. Lu, J. Zhou, C. Burrow, P. Wilson, Inhibition of HER-2(neu/ErbB2) restores normal function and structure to polycystic kidney disease (PKD) epithelia, Biochim. Biophys. Acta 1762 (2006) 647–655.
[49] W.E. Sweeney, L. Futey, P. Frost, E.D. Avner, In vitro modulation of cyst formation by a novel tyrosine kinase inhibitor, Kidney Int. 56 (1999) 406–413.
[50] W. Sweeney, K. Hamahira, J. Sweeney, M. Garcia-Gatrell, P. Frost, E. Avner, Combination treatment of PKD utilizing dual inhibition of EGF-receptor activity and ligand bioavailability, Kidney Int. 64 (2003) 1310–1319.
[51] W.E. Sweeney, Y. Chen, K. Nakanishi, P. Frost, E.D. Avner, Treatment of polycystic kidney disease with a novel tyrosine kinase inhibitor, Kidney Int. 57 (2000) 33–40.
[52] V. Torres, W. Sweeney, X. Wang, Q. Qian, P. Harris, P. Frost, E. Avner, EGF receptor tyrosine kinase inhibition attenuates the development of PKD in Han:SPRD rats, Kidney Int. 64 (2003) 1573–1579.
[53] V. Torres, W. Sweeney, X. Wang, Q. Qian, P. Harris, P. Frost, E. Avner, Epidermal growth factor receptor tyrosine kinase inhibition is not protective in PCK rats, Kidney Int. 66 (2004) 1766–1773.
[54] J.M. Shillingford, N.S. Murcia, C.H. Larson, S.H. Low, R. Hedgepeth, N. Brown, C.A. Flask, A.C. Novick, D.A. Goldfarb, A. Kramer-Zucker, G. Walz, K.B. Piontek, G.G. Germino, T. Weimbs, The mTOR pathway is regulated by polycystin-1, and its inhibition reverses renal cystogenesis in polycystic kidney disease, Proc. Natl Acad. Sci. USA 103 (2006) 5466–5471.
[55] A. Boletta, Emerging evidence of a link between the polycystins and the mTOR pathways, PathoGenetics 2 (2009) 6.
[56] J. Huang, B.D. Manning, The TSC1–TSC2 complex: a molecular switchboard controlling cell growth, Biochem. J. 412 (2008) 179–190.
[57] K.G. Foster, D.C. Fingar, Mammalian target of rapamycin (mTOR): conducting the cellular signaling symphony, J. Biol. Chem. 285 (2010) 14071–14077.
[58] N. Gao, D.C. Flynn, Z. Zhang, X.-S. Zhong, V. Walker, K.J. Liu, X. Shi, B.-H. Jiang, G1 cell cycle progression and the expression of G1 cyclins are regulated by PI3K/AKT/mTOR/p70S6K1 signaling in human ovarian cancer cells, Am. J. Physiol. Cell Physiol. 287 (2004) C281–C291.
[59] G. Distefano, M. Boca, I. Rowe, W. Claas, L. Ma, K. Piontek, G. Germino, P. Pandolfi, A. Boletta, Polycystin-1 regulates ERKs-dependent phosphorylation of Tuberin to control cell size through mTOR and its downstream effectors S6K and 4EBP1, Mol. Cell. Biol. (2009).
[60] R. Dere, P.D. Wilson, R.N. Sandford, C.L. Walker, Carboxy terminal tail of polycystin-1 regulates localization of TSC2 to repress mTOR, PLoS ONE 5 (2010) e9239.
[61] E. Kleymenova, O. Ibraghimov-Beskrovnaya, H. Kugoh, J. Everitt, H. Xu, K. Kiguchi, G. Landes, P. Harris, C. Walker, Tuberin-dependent membrane localization of polycystin-1: a functional link between polycystic kidney disease and the TSC2 tumor suppressor gene, Mol. Cell 7 (2001) 823–832.
[62] E. Meijer, P. de Jong, D. Peters, R. Gansevoort, Better understanding of ADPKD results in potential new treatment options: ready for the cure? J. Nephrol. 21 (2008) 133–138.
[63] V.H. Gattone, N.X. Chen, R.M. Sinders, M.F. Seifert, D. Duan, D. Martin, C. Henley, S.M. Moe, Calcimimetic inhibits late-stage cyst growth in ADPKD, J. Am. Soc. Nephrol. 20 (2009) 1527–1532.
[64] V.E. Torres, P.C. Harris, Polycystic kidney disease: genes, proteins, animal models, disease mechanisms and therapeutic opportunities, J. Intern. Med. 261 (2007) 17–31.
[65] A.B. Chapman, Autosomal dominant polycystic kidney disease: time for a change? J. Am. Soc. Nephrol. 18 (2007) 1399–1407.
[66] J.M. Shillingford, K.B. Piontek, G.G. Germino, T. Weimbs, Rapamycin ameliorates PKD resulting from conditional inactivation of Pkd1, J. Am. Soc. Nephrol. 21 (2010) 489–497.
[67] W.E. Sweeney, R.O. von Vigier, P. Frost, E.D. Avner, Src inhibition ameliorates polycystic kidney disease, J. Am. Soc. Nephrol. 19 (2008) 1331–1341.
[68] N.O. Bukanov, L.A. Smith, K.W. Klinger, S.R. Ledbetter, O. Ibraghimov-Beskrovnaya, Long-lasting arrest of murine polycystic kidney disease with CDK inhibitor roscovitine, Nature 444 (2006) 949–952.
[69] V.E. Torres, Treatment strategies and clinical trial design in ADPKD, Adv. Chron. Kidney Dis. 17 (2010) 190–204.
[70] A.L. Serra, D. Poster, A.D. Kistler, F. Krauer, S. Raina, J. Young, K.M. Rentsch, K.S. Spanaus, O. Senn, P. Kristanto, H. Scheffel, D. Weishaupt, R.P. Wüthrich, Sirolimus and kidney growth in autosomal dominant polycystic kidney disease, N. Engl. J. Med. 363 (2010) 820–829.
[71] G. Walz, K. Budde, M. Mannaa, J. Nürnberger, C. Wanner, C. Sommerer, U. Kunzendorf, B. Banas, W.H. Hörl, N. Obermüller, W. Arns, H. Pavenstädt, J. Gaedeke, M. Büchert, C. May, H. Gschaidmeier, S. Kramer, K.-U. Eckardt, Everolimus in patients with autosomal dominant polycystic kidney disease, N. Engl. J. Med. 363 (2010) 830–840.
[72] N. Perico, L. Antiga, A. Caroli, P. Ruggenenti, G. Fasolini, M. Cafaro, P. Ondei, N. Rubis, O. Diadei, G. Gherardi, S. Prandini, A. Panozo, R.F. Bravo, S. Carminati, F.R. De Leon, F. Gaspari, M. Cortinovis, N. Motterlini, B. Ene-Iordache, A. Remuzzi, G. Remuzzi, Sirolimus therapy to halt the progression of ADPKD, J. Am. Soc. Nephrol. 21 (2010) 1031–1040.
[73] T. Watnick, G.G. Germino, mTOR inhibitors in polycystic kidney disease, N. Engl. J. Med. 363 (2010) 879–881.
[74] N. Perico, G. Remuzzi, Polycystic kidney disease: do mTOR inhibitors still have a future in ADPKD? Nat. Rev. Nephrol. 6 (2010) 696–698.
[75] M.C. Hogan, T.V. Masyuk, L.J. Page, V.J. Kubly, E.J. Bergstralh, X. Li, B. Kim, B.F. King, J. Glockner, D.R. Holmes, S. Rossetti, P.C. Harris, N.F. LaRusso, V.E. Torres, Randomized clinical trial of long-acting somatostatin for autosomal dominant polycystic kidney and liver disease, J. Am. Soc. Nephrol. 21 (2010) 1052–1061.
[76] G.J. Pazour, B.L. Dickert, Y. Vucica, E.S. Seeley, J.L. Rosenbaum, G.B. Witman, D.G. Cole, Chlamydomonas IFT88 and its mouse homologue, polycystic kidney disease gene tg737, are required for assembly of cilia and flagella, J. Cell Biol. 151 (2000) 709–718.
[77] X. Hou, M. Mrug, B.K. Yoder, E.J. Lefkowitz, G. Kremmidiotis, P. D'Eustachio, D.R. Beier, L.M. Guay-Woodford, Cystin, a novel cilia-associated protein, is disrupted in the cpk mouse model of polycystic kidney disease, J. Clin. Invest. 109 (2002) 533–540.
[78] B.K. Yoder, X. Hou, L.M. Guay-Woodford, The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia, J. Am. Soc. Nephrol. 13 (2002) 2508–2516.
[79] J.L. Badano, N. Mitsuma, P.L. Beales, N. Katsanis, The ciliopathies: an emerging class of human genetic disorders, Annu. Rev. Genomics Hum. Genet. 7 (2006) 125–148.
[80] M.A. Lancaster, J.G. Gleeson, The primary cilium as a cellular signaling center: lessons from disease, Curr. Opin. Genet. Dev. 19 (2009) 220–229.
[81] K. Baker, P.L. Beales, Making sense of cilia in disease: the human ciliopathies, Am. J. Med. Genet. C Semin. Med. Genet. 151C (2009) 281–295.
[82] C. Deltas, G. Papagregoriou, Cystic diseases of the kidney: molecular biology and genetics, Arch. Pathol. Lab. Med. 134 (2010) 569–582.
[83] B.K. Yoder, Role of primary cilia in the pathogenesis of polycystic kidney disease, J. Am. Soc. Nephrol. 18 (2007) 1381–1388.
[84] V. Singla, J.F. Reiter, The primary cilium as the cell's antenna: signaling at a sensory organelle, Science 313 (2006) 629–633.
[85] H.A. Praetorius, K.R. Spring, Bending the MDCK cell primary cilium increases intracellular calcium, J. Membr. Biol. 184 (2001) 71–79.
[86] S.M. Nauli, F.J. Alenghat, Y. Luo, E. Williams, P. Vassilev, X. Li, A.E.H. Elia, W. Lu, E.M. Brown, S.J. Quinn, D.E. Ingber, J. Zhou, Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells, Nat. Genet. 33 (2003) 129–137.
[87] S. Wang, J. Zhang, S.M. Nauli, X. Li, P.G. Starremans, Y. Luo, K.A. Roberts, J. Zhou, Fibrocystin/polyductin, found in the same protein complex with polycystin-2, regulates calcium responses in kidney epithelia, Mol. Cell. Biol. 27 (2007) 3241–3252.
[88] S.H. Low, S. Vasanth, C.H. Larson, S. Mukherjee, N. Sharma, M.T. Kinter, M.E. Kane, T. Obara, T. Weimbs, Polycystin-1, STAT6, and P100 function in a pathway that transduces ciliary mechanosensation and is activated in polycystic kidney disease, Dev. Cell 10 (2006) 57–69.
[89] J.R. Davenport, A.J. Watts, V.C. Roper, M.J. Croyle, T. van Groen, J.M. Wyss, T.R. Nagy, R.A. Kesterson, B.K. Yoder, Disruption of intraflagellar transport in adult mice leads to obesity and slow-onset cystic kidney disease, Curr. Biol. 17 (2007) 1586–1594.
[90] I.S. Lantinga-van Leeuwen, W.N. Leonhard, A. van der Wal, M.H. Breuning, E. de Heer, D.J.M. Peters, Kidney-specific inactivation of the Pkd1 gene induces rapid cyst formation in developing kidneys and a slow onset of disease in adult mice, Hum. Mol. Genet. 16 (2007) 3188–3196.
[91] K. Piontek, L.F. Menezes, M.A. Garcia-Gonzalez, D.L. Huso, G.G. Germino, A critical developmental switch defines the kinetics of kidney cyst formation after loss of Pkd1, Nat. Med. 13 (2007) 1490–1495.
[92] A. Takakura, L. Contrino, A.W. Beck, J. Zhou, Pkd1 inactivation induced in adulthood produces focal cystic disease, J. Am. Soc. Nephrol. 19 (2008) 2351–2363.
[93] V. Patel, L. Li, P. Cobo-Stark, X. Shao, S. Somlo, F. Lin, P. Igarashi, Acute kidney injury and aberrant planar cell polarity induce cyst formation in mice lacking renal cilia, Hum. Mol. Genet. 17 (2008) 1578–1590.
[94] H. Happé, W.N. Leonhard, A. van der Wal, B. van de Water, I.S. Lantinga-van Leeuwen, M.H. Breuning, E. de Heer, D.J.M. Peters, Toxic tubular injury in kidneys from Pkd1-deletion mice accelerates cystogenesis accompanied by dysregulated planar cell polarity and canonical Wnt signaling pathways, Hum. Mol. Genet. 18 (2009) 2532–2542.
[95] F. Verdeguer, S. Le Corre, E. Fischer, C. Callens, S. Garbay, A. Doyen, P. Igarashi, F. Terzi, M. Pontoglio, A mitotic transcriptional switch in polycystic kidney disease, Nat. Med. 16 (2010) 106–110.
[96] T.J. Klein, M. Mlodzik, Planar cell polarization: an emerging model points in the right direction, Annu. Rev. Cell Dev. Biol. 21 (2005) 155–176.
[97] M.A. Lancaster, C.M. Louie, J.L. Silhavy, L. Sintasath, M. Decambre, S.K. Nigam, K. Willert, J.G. Gleeson, Impaired Wnt-beta-catenin signaling disrupts adult renal homeostasis and leads to cystic kidney ciliopathy, Nat. Med. 15 (2009) 1046–1054.
[98] M. Simons, J. Gloy, A. Ganner, A. Bullerkotte, M. Bashkurov, C. Krönig, B. Schermer, T. Benzing, O.A. Cabello, A. Jenny, M. Mlodzik, B. Polok, W. Driever, T. Obara, G. Walz, Inversin, the gene product mutated in nephronophthisis type II,

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functions as a molecular switch between Wnt signaling pathways, Nat. Genet. 37 (2005) 537–543.
[99] L. Gresh, E. Fischer, A. Reimann, M. Tanguy, S. Garbay, X. Shao, T. Hiesberger, L. Fiette, P. Igarashi, M. Yaniv, M. Pontoglio, A transcriptional network in polycystic kidney disease, EMBO J. 23 (2004) 1657–1668.
[100] T.V. Masyuk, B.Q. Huang, A.I. Masyuk, E.L. Ritman, V.E. Torres, X. Wang, P.C. Harris, N.F. LaRusso, Biliary dysgenesis in the PCK rat, an orthologous model of autosomal recessive polycystic kidney disease, Am. J. Pathol. 165 (2004) 1719–1730.
[101] E. Fischer, E. Legue, A. Doyen, F. Nato, J.-F. Nicolas, V. Torres, M. Yaniv, M. Pontoglio, Defective planar cell polarity in polycystic kidney disease, Nat. Genet. 38 (2006) 21–23.
[102] S. Saburi, I. Hester, E. Fischer, M. Pontoglio, V. Eremina, M. Gessler, S.E. Quaggin, R. Harrison, R. Mount, H. McNeill, Loss of Fat4 disrupts PCP signaling and oriented cell division and leads to cystic kidney disease, Nat. Genet. 40 (2008) 1010–1015.
[103] T.J. Park, S.L. Haigo, J.B. Wallingford, Ciliogenesis defects in embryos lacking inturned or fuzzy function are associated with failure of planar cell polarity and Hedgehog signaling, Nat. Genet. 38 (2006) 303–311.
[104] A. Borovina, S. Superina, D. Voskas, B. Ciruna, Vangl2 directs the posterior tilting and asymmetric localization of motile primary cilia, Nat. Cell Biol. 12 (2010) 407–412.
[105] F. Kotsis, R. Nitschke, M. Doerken, G. Walz, E.W. Kuehn, Flow modulates centriole movements in tubular epithelial cells, Pflugers Arch 456 (2008) 1025–1035.
[106] B. Guirao, A. Meunier, S. Mortaud, A. Aguilar, J.-M. Corsi, L. Strehl, Y. Hirota, A. Desoeuvre, C. Boutin, Y.-G. Han, Z. Mirzadeh, H. Cremer, M. Montcouquiol, K. Sawamoto, N. Spassky, Coupling between hydrodynamic forces and planar cell polarity orients mammalian motile cilia, Nat. Cell Biol. 12 (2010) 341–350.
[107] C.M. Karner, R. Chirumamilla, S. Aoki, P. Igarashi, J.B. Wallingford, T.J. Carroll, Wnt9b signaling regulates planar cell polarity and kidney tubule morphogenesis, Nat. Genet. 41 (2009) 793–799.
[108] S. Nishio, X. Tian, A.R. Gallagher, Z. Yu, V. Patel, P. Igarashi, S. Somlo, Loss of oriented cell division does not initiate cyst formation, J. Am. Soc. Nephrol. (2009).
[109] L.B. Pedersen, J.L. Rosenbaum, Intraflagellar transport (IFT) role in ciliary assembly, resorption and signalling, Curr. Top. Dev. Biol. 85 (2008) 23–61.
[110] J.M. Gerdes, Y. Liu, N.A. Zaghloul, C.C. Leitch, S.S. Lawson, M. Kato, P.A. Beachy, P.L. Beales, G.N. DeMartino, S. Fisher, J.L. Badano, N. Katsanis, Disruption of the basal body compromises proteasomal function and perturbs intracellular Wnt response, Nat. Genet. 39 (2007) 1350–1360.
[111] S. Doxsey, W. Zimmerman, K. Mikule, Centrosome control of the cell cycle, Trends Cell Biol. 15 (2005) 303–311.
[112] I. Hoffmann, Linking the cell cycle to the centrosome cycle, 2005.
[113] P. Meraldi, E. Nigg, The centrosome cycle, FEBS Lett. 521 (2002) 9–13.
[114] J.A. Follit, R.A. Tuft, K.E. Fogarty, G.J. Pazour, The intraflagellar transport protein IFT20 is associated with the Golgi complex and is required for cilia assembly, Mol. Biol. Cell 17 (2006) 3781–3792.
[115] A. Jurczyk, A. Gromley, S. Redick, J. San Agustin, G. Witman, G.J. Pazour, D.J.M. Peters, S. Doxsey, Pericentrin forms a complex with intraflagellar transport proteins and polycystin-2 and is required for primary cilia assembly, J. Cell Biol. 166 (2004) 637–643.
[116] A. Robert, G. Margall-Ducos, J.-E. Guidotti, O. Brégerie, C. Celati, C. Bréchot, C. Desdouets, The intraflagellar transport component IFT88/polaris is a centrosomal protein regulating G1–S transition in non-ciliated cells, J. Cell Sci. 120 (2007) 628–637.
[117] V. Srsen, N. Gnadt, A. Dammermann, A. Merdes, Inhibition of centrosome protein assembly leads to p53-dependent exit from the cell cycle, J. Cell Biol. 174 (2006) 625–630.
[118] K. Mikule, B. Delaval, P. Kaldis, A. Jurcyzk, P. Hergert, S. Doxsey, Loss of centrosome integrity induces p38–p53–p21-dependent G1–S arrest, Nat. Cell Biol. 9 (2007) 160–170.
[119] K. Fukasawa, T. Choi, R. Kuriyama, S. Rulong, G. Vande Woude, Abnormal centrosome amplification in the absence of p53, Science 271 (1996) 1744–1747.
[120] P. Tarapore, K. Fukasawa, Loss of p53 and centrosome hyperamplification, Oncogene 21 (2002) 6234–6240.
[121] P. Tarapore, H. Horn, Y. Tokuyama, K. Fukasawa, Direct regulation of the centrosome duplication cycle by the p53-p21Waf1/Cip1 pathway, Oncogene 20 (2001) 3173–3184.
[122] K. Shinmura, R. Bennett, P. Tarapore, K. Fukasawa, Direct evidence for the role of centrosomally localized p53 in the regulation of centrosome duplication, Oncogene 26 (2007) 2939–2944.
[123] A. Bhunia, K. Piontek, A. Boletta, L. Liu, F. Qian, P. Xu, F. Germino, G. Germino, PKD1 induces p21(waf1) and regulation of the cell cycle via direct activation of the JAK-STAT signaling pathway in a process requiring PKD2, Cell 109 (2002) 157–168.
[124] X. Li, Y. Luo, P.G. Starremans, C.A. McNamara, Y. Pei, J. Zhou, Polycystin-1 and polycystin-2 regulate the cell cycle through the helix–loop–helix inhibitor Id2, Nat. Cell Biol. 7 (2005) 1202–1212.
[125] H. Kim, Y. Bae, W. Jeong, C. Ahn, S. Kang, Depletion of PKD1 by an antisense oligodeoxynucleotide induces premature G1/S-phase transition, Eur. J. Hum. Genet. 12 (2004) 433–440.
[126] L. Battini, E. Fedorova, S. Macip, X. Li, P.D. Wilson, G.L. Gusella, Stable knockdown of polycystin-1 confers integrin-α2β1-mediated anoikis resistance, J. Am. Soc. Nephrol. 17 (2006) 3049–3058.
[127] S. Nishio, M. Hatano, M. Nagata, S. Horie, T. Koike, T. Tokuhisa, T. Mochizuki, Pkd1 regulates immortalized proliferation of renal tubular epithelial cells through p53 induction and JNK activation, J. Clin. Invest. 115 (2005) 910–918.
[128] L. Battini, S. Macip, E. Fedorova, S. Dikman, S. Somlo, C. Montagna, G.L. Gusella, Loss of polycystin-1 causes centrosome amplification and genomic instability, Hum. Mol. Genet. 17 (2008) 2819–2833.
[129] M. Castedo, J. Perfettini, T. Roumier, K. Andreau, R. Medema, G. Kroemer, Cell death by mitotic catastrophe: a molecular definition, Oncogene 23 (2004) 2825–2837.
[130] M. Castedo, J. Perfettini, T. Roumier, A. Valent, H. Raslova, K. Yakushijin, D. Horne, J. Feunteun, G. Lenoir, R. Medema, W. Vainchenker, G. Kroemer, Mitotic catastrophe constitutes a special case of apoptosis whose suppression entails aneuploidy, Oncogene 23 (2004) 4362–4370.
[131] S. Burtey, M. Riera, E. Ribe, P. Pennenkamp, R. Rance, J. Luciani, B. Dworniczak, M.G. Mattei, M. Fontés, Centrosome overduplication and mitotic instability in PKD2 transgenic lines, Cell Biol. Int. 32 (2008) 1193–1198.
[132] J. Zhang, M. Wu, S. Wang, J. Shah, P.D. Wilson, J. Zhou, Polycystic kidney disease protein fibrocystin localizes to the mitotic spindle and regulates spindle bipolarity, Hum. Mol. Genet. (2010).
[133] D.R. Rundle, G. Gorbsky, L. Tsiokas, PKD2 interacts and co-localizes with mDia1 to mitotic spindles of dividing cells: role of mDia1 IN PKD2 localization to mitotic spindles, J. Biol. Chem. 279 (2004) 29728–29739.
[134] A.F. Palazzo, T.A. Cook, A.S. Alberts, G.G. Gundersen, mDia mediates Rho-regulated formation and orientation of stable microtubules, Nat. Cell Biol. 3 (2001) 723–729.
[135] Q. Li, N. Montalbetti, P.Y. Shen, X.-Q. Dai, C.I. Cheeseman, E. Karpinski, G. Wu, H.F. Cantiello, X.-Z. Chen, Alpha-actinin associates with polycystin-2 and regulates its channel activity, Hum. Mol. Genet. 14 (2005) 1587–1603.
[136] Q. Li, N. Montalbetti, Y. Wu, A. Ramos, M.K. Raychowdhury, X.-Z. Chen, H.F. Cantiello, Polycystin-2 cation channel function is under the control of microtubular structures in primary cilia of renal epithelial cells, J. Biol. Chem. 281 (2006) 37566–37575.
[137] K. Hanaoka, F. Qian, A. Boletta, A. Bhunia, K. Piontek, L. Tsiokas, V. Sukhatme, W. Guggino, G. Germino, Co-assembly of polycystin-1 and -2 produces unique cation-permeable currents, Nature 408 (2000) 990–994.
[138] L. De Boer, V. Oakes, H. Beamish, N. Giles, F. Stevens, M. Somodevilla-Torres, C. Desouza, B. Gabrielli, Cyclin A/cdk2 coordinates centrosomal and nuclear mitotic events, Oncogene 27 (2008) 4261–4268.
[139] F. Mitsuyama, T. Sawai, The redistribution of $Ca^{2+}$ stores with inositol 1,4,5-trisphosphate receptor to the cleavage furrow in a microtubule-dependent manner, Int. J. Dev. Biol. 45 (2001) 861–868.
[140] Y. Matsumoto, J.L. Maller, Calcium, calmodulin, and CaMKII requirement for initiation of centrosome duplication in *Xenopus* egg extracts, Science 295 (2002) 499–502.
[141] Q. Qian, M. Li, Y. Cai, C. Ward, S. Somlo, P. Harris, V. Torres, Analysis of the polycystins in aortic vascular smooth muscle cells, J. Am. Soc. Nephrol. 14 (2003) 2280–2287.
[142] G.I. Anyatonwu, M. Estrada, X. Tian, S. Somlo, B.E. Ehrlich, Regulation of ryanodine receptor-dependent calcium signaling by polycystin-2, Proc. Natl Acad. Sci. USA 104 (2007) 6454–6459.
[143] G. Aguiari, V. Trimi, M. Bogo, A. Mangolini, G. Szabadkai, P. Pinton, R. Witzgall, P. C. Harris, P.A. Borea, R. Rizzuto, L. del Senno, Novel role for polycystin-1 in modulating cell proliferation through calcium oscillations in kidney cells, Cell Prolif. 41 (2008) 554–573.
[144] P. Uhlén, N. Fritz, Biochemistry of calcium oscillations, Biochem. Biophys. Res. Commun. 396 (2010) 28–32.
[145] A. Astrinidis, W. Senapedis, E.P. Henske, Hamartin, the tuberous sclerosis complex 1 gene product, interacts with polo-like kinase 1 in a phosphorylation-dependent manner, Hum. Mol. Genet. 15 (2006) 287–297.
[146] R. Tammachote, C.J. Hommerding, R.M. Sinders, C.A. Miller, P.G. Czarnecki, A.C. Leightner, J.L. Salisbury, C.J. Ward, V.E. Torres, V.H. Gattone, P.C. Harris, Ciliary and centrosomal defects associated with mutation and depletion of the Meckel syndrome genes MKS1 and MKS3, Hum. Mol. Genet. 18 (2009) 3311–3323.
[147] J.A. Jonassen, J. San Agustin, J.A. Follit, G.J. Pazour, Deletion of IFT20 in the mouse kidney causes misorientation of the mitotic spindle and cystic kidney disease, J. Cell Biol. 183 (2008) 377–384.
[148] J.L. Brasier, E.P. Henske, Loss of the polycystic kidney disease (PKD1) region of chromosome 16p13 in renal cyst cells supports a loss-of-function model for cyst pathogenesis, J. Clin. Invest. 99 (1997) 194–199.
[149] J. Gogusev, I. Murakami, M. Doussau, L. Telvi, A. Stojkoski, P. Lesavre, D. Droz, Molecular cytogenetic aberrations in autosomal dominant polycystic kidney disease tissue, J. Am. Soc. Nephrol. 14 (2003) 359–366.
[150] W.F. Marshall, Basal bodies platforms for building cilia, Curr. Top. Dev. Biol. 85 (2008) 1–22.

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